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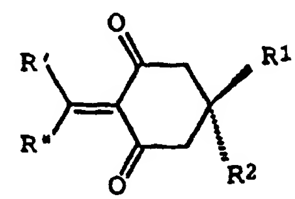
(54) Title: OLIGOSACCHARIDE SYNTHESIS

(57) Abstract

The invention provides a system for solid-phase synthesis of oligosaccharides, based on the discovery that a 2-substituted-1,3-dioxocycloalkyl linker group of general formula (I) can be used to couple saccharide groups of both the O-glycoside and N-glycoside type to a polymer support. The invention provides reagents, reagent kits and methods for solid-phase oligosaccharide synthesis.



(I)

OLIGOSACCHARIDE SYNTHESIS

FIELD OF THE INVENTION

This invention relates to methods for synthesis of oligosaccharides, and in particular to methods for solid phase or combinatorial synthesis of oligosaccharides. The invention provides a novel linker-resin, linker-saccharide, or resin-linker-saccharide complex, which in one embodiment enables a saccharide residue to be linked to a soluble or insoluble polymeric support for use as a basis for solid-phase synthesis of oligosaccharides. In a second embodiment, the complex of the invention enables oligosaccharides to be linked to a solid polymeric support for use as an analytical reagent.

BACKGROUND OF THE INVENTION

It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art, in Australia or in any other country.

Oligosaccharides constitute a major class of bioactive polymers, implicated in biochemical processes (Lasky, 1992; Varki, 1993) as diverse as cellular differentiation, hormone-cell recognition and cell-cell adhesion, especially viral-host cell (Gambaryan *et al*, 1995) and bacteria-host cell attachment (Boren *et al*, 1993). Involvement of oligosaccharides in diseases such as cancer, cardiovascular disorders, microbial infections, graft rejection and autoimmune disorders has therefore, been strongly suggested. Conjugation of carbohydrates to bioactive peptides has also been demonstrated to stabilise the peptides against degradation, and, in more specific circumstances, to facilitate peptide transport across biological barriers (Lee, 1989; Fisher, 1991; Rodriguez, 1989). Thus the ability to synthesise oligosaccharides in

a facile and efficient manner is now becoming an extremely important area within organic chemistry.

The highly labour intensive solution phase strategies hitherto utilised in oligosaccharide syntheses require an extremely specialised knowledge and a high degree of chemical skill. This situation was mirrored

within the area of peptide synthesis, until Merrifield *et al* proposed and developed Solid Phase Peptide Synthesis (SPPS) over thirty years ago (Merrifield, 1963). In SPPS immobilisation of the first amino acid of the required sequence to an insoluble resin enabled large excesses of reagents to be used to achieve the coupling of the second amino acid. Any unused materials remaining at the end of the coupling step could then be removed simply by washing the resin beads. This technology meant that the chemist could drive each coupling reaction to almost quantitative yields, and since the peptide intermediates formed were still bound to the resin, purification after each acylation step was not required. SPPS enables peptide and polypeptide synthesis to be employed as a routine research and synthetic tool, and permits large-scale combinatorial synthesis of peptides for screening of potential pharmaceutical agents.

For many years chemists have attempted to transpose this solid-phase methodology to oligosaccharide synthesis, with varying degrees of success. The first attempt was approximately 25 years ago (Frechet and Schuerch, 1971; Frechet and Schuerch, 1972; Guthrie *et al*, 1971; Guthrie *et al*, 1973). However, the ozone-mediated deprotection product was an aldehyde-substituted glycoside. Danishefsky and coworkers described the solid phase synthesis of the Lewis b Antigen (Randolph *et al*, 1995) and *N*-linked glycopeptides (Roberge *et al*, 1995) by initial attachment of the primary sugar unit of the oligosaccharide to a 1% divinylbenzene-styrene co-polymer support *via* a silyl ether linkage. The resin-bound sugar moeity was in this instance a glycal, with on-resin activation achieved via epoxidation of the double bond, and the resulting glycal residue acting as a sugar donor through nucleophile ring-opening of the epoxide. Since there are no colorimetric methods available to the sugar chemist to monitor on-resin glycosylations, the only means of assessing the progress of the reaction is by lysis of the

oligosaccharide-resin bond and subsequent analysis of the cleavage product, usually by thin layer chromatography. The tetra-n-butylammonium fluoride-mediated deprotection conditions required to cleave Danishefsky's silyl ether linker are both hazardous and slow. This coupled with the requirement for on-resin activation of the tethered glycals, makes the overall strategy and methodology far from ideal.

In an alternative approach, Douglas and coworkers described the synthesis of D-mannopentose using a polyethyleneglycol ω-monomethylether co-polymer and a succinoyl or an α,α'-dioxyxylyl diether linker (Douglas *et al*, 1995). The reactions were carried out in solution phase, with removal of unused reactants being achieved by precipitation of the oligosaccharide-polymer complex and subsequent washing. In the latter example, cleavage of the oligosaccharide-polymer bond was achieved through catalytic hydrogenation, which required exposure of the conjugate to 1 atm of H_2 for 48 h to achieve respectable yields. This again is far too slow to allow effective monitoring of individual glycosylation reactions. Yan *et al* reported sulphoxide-mediated glycosylation on a Merrifield resin, using a thiophenol linker for the attachment of the primary sugar residue (Yan *et al*, 1994). This method resulted in the construction of (1-6)-linked oligosaccharides, and was suitable for synthesis of both α- and β-glycosidic linkages. However, the thioglycosidic linkage to the resin dictates that similar sugar donors cannot be employed in this strategy.

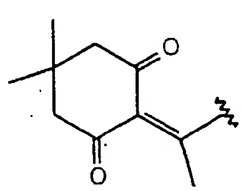
Recently Rademann and Schmidt reported the use of trichloroacetimidate sugar donors to a resin bound sugar tethered *via* an alkyl thiol (Rademann and Schmidt, 1996); once again, however, this method precludes the use of the far superior thioglycoside sugar donors. Meanwhile, Adinolfi *et al* described the synthesis of disaccharides using a polyethyleneglycol-polystyrene resin, with connection of the first sugar to the polymeric support

through a succinate spacer (Adinolfi *et al*, 1996). However, the acid lability displayed by this linker means that the primary sugar cannot be linked to the resin *via* the glycosidic position.

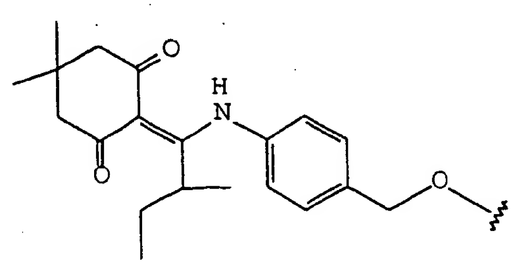
The above examples serve to illustrate that the critical element in solid phase synthesis is the nature of the linker between the solid support and the initial synthon. The linker must display excellent stability to the conditions of coupling and deprotection, yet in the case of solid phase oligosaccharide synthesis, it should also be rapidly and efficiently cleaved to allow monitoring of the progress of individual coupling reactions. The cleavage should ideally be achieved by the use of a relatively innocuous chemical reagent.

It is clear, then, that there remains a need in the art for simple, efficient and economical methods for solid-phase synthesis of oligosaccharides.

A hydrazine-labile primary amino-protecting group, *N*-1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde), has been reported for protection of lysine side chains during SPPS (Bycroft *et al*, 1993). This group was modified for use as a carboxy-protecting group in SPPS when the 2-(3-methylbutyryl)dimedone analogue of 2-acetyl-dimedone was condensed with 4-aminobenzylalcohol to afford 4-[*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methyl-butyl]-amino]benzyl ester (ODmab)(Chan *et al*, 1995).



Dde



ODmab

The two protecting groups were reported to be stable to the deprotecting conditions widely used in SPPS, *ie.* trifluoroacetic acid (TFA) or 20% piperidine in dimethyl formamide (DMF). The ethyl ester, 4-[N-(1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl)amino]benzyl ester (ODab) showed small but significant instability to 20% piperidine-DMF. Both Dde and ODmab are linked to groups on amino acids, rather than directly to the solid-phase support. Their use in solid-phase oligosaccharide synthesis has not been suggested.

We have now surprisingly found that protecting groups similar to Dde and ODmab can be coupled to a polymeric support, thereby generating a system for the immobilisation of sugars. To this end we have immobilised *N*- and *O*-glycosides to the solid support and synthesised oligosaccharides using various sugar donors. The linkers display excellent stability to most acids and secondary/tertiary bases encountered in modern synthetic chemistry, yet are rapidly and efficiently cleaved with either ammonia, hydrazine or primary amines.

Bannwarth *et al* have independently developed a different solid phase linker around the Dde protecting group, which they have utilised for the immobilisation of amino acids and primary amines for combinatorial library synthesis (Bannwarth *et al*, 1996). However, the synthesis of this linker is both protracted and inefficient, and the linker only displays a limited stability to secondary bases such as piperidine. There has been no suggestion that this linker could be used for oligosaccharide synthesis. The linkers we have developed demonstrate a far greater stability than those of Bannwarth *et al*.

SUMMARY OF THE INVENTION

In one aspect, the invention provides a support for solid-phase synthesis of oligosaccharides, said support comprising:

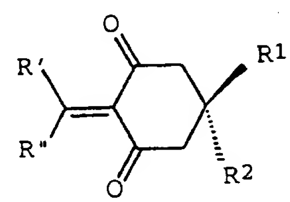
a) a resin,

b) a linker covalently attached to the resin, and

c) one or more saccharide groups covalently attached to the resin via the linker,

wherein the linker is a 2-substituted-1,3-dioxocycloalkane compound, and

said support having general formula I:



I

in which

R^1 and R^2 may be the same or different, and is each hydrogen or C_{1-4} alkyl;

R' is an amino sugar, a glycosylamine, or a glycosylamine of an oligosaccharide; a mono or oligosaccharide coupled through an alkyl-, substituted alkyl-, aryl-, substituted aryl-, cycloalkyl-, or substituted cycloalkyl-amino group; or a mono or oligosaccharide coupled through a carboxyalkyl-, substituted carboxyalkyl-, carboxyaryl-, substituted carboxyaryl-, carboxycycloalkyl-, or substituted carboxycycloalkyl-amino group; and

R" is an alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, or substituted cycloalkyl spacer group which is directly coupled to the resin support, or which may optionally be coupled to the resin support via a suitable covalent linkage, which is stable to conditions of oligosaccharide synthesis and cleavage.

The covalent linkage to the resin may suitably be provided by a -CONH-, -O-, -S-, -COO-, -CH=N-, -NHCONH-, -NHCSNH, or -NHNH- grouping, *eg.* Spacer-CONH-resin, Spacer-

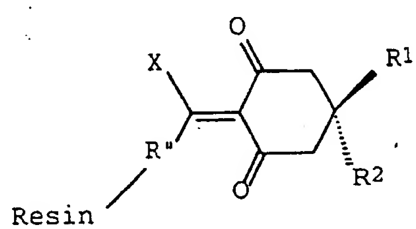
O-resin, Spacer-S-resin, Spacer-CO_2-resin, Spacer-CH=N-resin, Spacer-NHCONH-resin, Spacer-NHCSNH-resin, Spacer-NHNH-resin. Other possible covalent linking groups will be known to those skilled in the art.

Preferably both R^1 and R^2 are methyl.

Preferably R' is an oligosaccharide-O-CH_2-(C_6H_4)-NH, monosaccharide-O-CH_2-(C_6H_4)-NH, amino-oligosaccharide-CO_2CH_2-(C_6H_4)NH, or amino-monosaccharide-CO_2CH_2-(C_6H_4)-NH group.

In a particularly preferred embodiment the 2-substituted-1,3-dioxocycloalkane linker is functionalised Dde, Ddh or ODmab. In one very particularly preferred embodiment the support comprises a resin, a linker and a monosaccharide, an oligosaccharide, an aminosaccharide or an amino-oligosaccharide.

In a second aspect, the invention provides a support for solid-phase synthesis comprising a resin and a linker group, wherein the linker is a 2-substituted-1,3-dioxocycloalkane of general formula II:



II

in which

X is OH or NH_2;

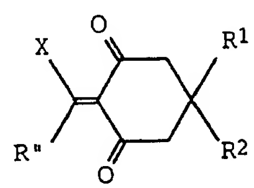
R^1 and R^2 may be the same or different, and is each hydrogen or C_{1-4} alkyl; preferably both R^1 and R^2 are methyl; and

R" is an alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, or substituted cycloalkyl spacer group which is directly coupled to the resin

support, or which may optionally be coupled to the resin support via a suitable covalent linkage, which is stable to conditions of oligosaccharide synthesis and cleavage. The covalent linkage may suitably be provided by a -CONH-, -O-, -S-, -COO-, -CH=N-, -NHCONH-, -NHCSNH, or -NHNH- grouping, *eg*. Spacer-CONH-resin, Spacer-O-resin, Spacer-S-resin, Spacer-CO_2-resin, Spacer-CH=N-resin, Spacer-NHCONH-resin, Spacer-NHCSNH-resin, Spacer-NHNH-resin. Other possible covalent linking groups will be known to those skilled in the art.

In a third aspect, the invention provides a linker-saccharide complex, comprising a linker group of general formula II as defined above and a saccharide group as defined above for R'.

In a fourth aspect the invention provides a linker compound carrying functional groups suitable to attach a primary amine to a resin via covalent bonds which are stable to conditions of oligosaccharide synthesis and cleavage, said compound having general formula III



III

in which

X is OH or NH_2;

R^1 and R^2 may be the same or different, and is each hydrogen or C_{1-4} alkyl, and

R" is an alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, or substituted cycloalkyl spacer group, which carries a functionality capable of reacting with a functionalised resin.

Preferably the linker compound is 6-hydroxyl-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid or an ester thereof. Preferably the ester is a benzyl, methyl, or t-butyl ester.

For the purposes of this specification the term "substituted" in the definitions of substituents within this specification means that the substituent is itself substituted with a group which does not change the general chemical characteristics of the substituent. Preferred such further substituents are halogen, nitro, amino, hydroxyl, and thiol; preferred halogens are chlorine and iodine. The person skilled in the art will be aware of other suitable substituents of similar size and charge characteristics which could be used as alternatives in a given situation.

For the purposes of this specification a compound is regarded as "stable to conditions of oligosaccharide synthesis and cleavage" if there is less than 10% loss of the compound after exposure at room temperature to ammonia, hydrazine or a primary amino compound in water or DMF. The person skilled in the art will readily be able to determine whether the stability of a particular compound is adequate for it to be useful for the purposes of the invention, using conditions appropriate for his or her particular needs.

For the purposes of this specification it will be clearly understood that the word "comprising" means "including but not limited to", and that the word "comprises" has a corresponding meaning.

The linker compound of the invention may be synthesized on the resin, or may be synthesized in solution.

The invention also provides kits useful in solid phase synthesis or combinatorial synthesis of oligosaccharides, comprising either

a) a resin-linker-saccharide support,

b) a linker-saccharide complex, or

c) a resin-linker support,

according to the invention, as described above. The kit may optionally also comprise one or more further reagents such as protecting agents, deprotecting agents, and/or

solvents suitable for solid phase or combinatorial synthesis. The person skilled in the art will be aware of suitable further reagents. Different types of kit can then be chosen according to the desired use.

The resin may be any resin which swells in water and/or in an organic solvent, and which comprises one of the following substituents: halogen, hydroxy, carboxyl, SH, NH_2, formyl, SO_2NH_2, or $NHNH_2$, for example methylbenzhydrylamine (MBHA) resin, amino or carboxy tentagel resins, 4-sulphamylbenzyl AM resin. Other suitable resins will be known to those skilled in the art.

The invention also provides a method of solid-phase synthesis of oligosaccharides, comprising the step of sequentially linking mono- or oligosaccharide groups to a support as described above. Similarly the mono- or oligosaccharide building blocks may be as described above.

This method is particularly useful for combinatorial synthetic application.

The linker compound may be synthesised in solution or directly on the resin in a stepwise manner prior to the coupling of the initial sugar group, or the linker-initial sugar conjugate may be synthesised in solution phase and subsequently coupled to the solid support, with subsequent sugars being sequentially attached. Preferably the second and all subsequent sugar groups are coupled to the oligosaccharide chain-resin conjugate after the last sugar in the oligosaccharide chain is partially deprotected.

The invention accordingly provides a method of synthesis of a linker group according to general formula I as defined above, comprising the step of C-acylation of a 2-substituted 1,3-dioxocyclohexane compound with a dicarboxylic acid. Preferably the dicarboxylic acid is mono-protected by ester formation. More preferably the reaction is activated with carbodiimide and catalysed by N,N'-dimethylaminopyridine.

The product of the reaction may optionally be reacted with 4-aminobenzyl alcohol, to form the 4-aminobenzyl derivative.

The invention also provides a method of synthesis of a resin-linker support, comprising the step of swelling a resin in a suitable solvent, treating the swollen resin with a dicarboxylic acid, and reacting the thus-produced product with a 2-substituted 1,3-dioxocycloalkane compound. Preferably for both synthesis of the linker and synthesis of the resin-linker support the 2-substituted 1,3-dioxocyclolkane compound is 5,5-dimethyl-1,3-cyclohexanedione. Also preferably the dicarboxylic acid is adipic acid.

The first sugars attached to the resin-linker unit may be unprotected, partially protected or fully protected glycosides, aminoglycosides, or ether- or amino-linked sugars, where the coupling takes place through a non-glycosidic position.

The building block mono- or oligosaccharide-donors may be any activated sugar, including but not limited to orthoesters, thioorthoesters, cyanoalkylidene derivatives, 1-*O*-acyl sugars, amino sugars, acetimidates, trichloroacetimidates, thioglycosides, aminoglycosides, amino-oligosaccharides, glycosylamines of oligosaccharides, glycosyl thiocyanates, pentenyl glycosides, pentenoylglycosides, isoprenyl glycosides, glycals, tetramethylphosphoro diamidates, sugar diazirines, selenoglycosides, phosphorodithioates, glycosyl-dialkylphosphites, glycosylsulphoxides and glycosylfluorides.

Preferably the first sugar coupled to the resin is an aminosugar, an aminoglycoside, or an amino-oligosaccharide or a glycosyl amine of an oligosaccharide.

Preferably partial sugar deprotection is achieved by using acyl-type, trityl, benzyl-type, acetal-type, or various silyl and/or photolabile protecting groups in addition to permanent protecting groups. This permits the

synthesis of branched oligosaccharides by using two orthogonal hydroxy-protecting groups on a single sugar donor.

The synthesised oligosaccharide can be cleaved from the resin using ammonia, hydrazine or a primary amine, such as butylamine or cyclohexylamine. For the preparation of aminoglycosides, ammonia or a suitable primary amine in an organic solvent is preferably employed. For the preparation of hydrazides, hydrazine in water or in an organic solvent is preferably employed. For the preparation of oligosaccharides, ammonia in water or in an organic solvent is preferably employed, followed by acidification. When the linker contains a 4-aminobenzyl moiety, after cleavage as described above the first sugar is released still protected by the aminobenzyl group; this can be removed by hydrogenation if desired.

The person skilled in the art will appreciate that the oligosaccharide can be retained on the resin for use as an analytical or preparative reagent, for example in affinity chromatography or for bulk-scale affinity separation.

Detailed Description of the Figures

Figure 1 shows a general representation of the strategy required for solid phase oligosaccharide synthesis.

Figure 2 illustrates a general representation of the 'divide-couple-recombine' method of oligosaccharide library synthesis utilising a solid phase strategy.

Figure 3 shows the synthesis of the Dde-based linker of the invention, attachment of the primary sugar residue and coupling of the sugar-linker conjugate to a resin support. An alternative approach whereby the linker is synthesised directly on the resin is also shown.

Figure 4 shows the synthesis of the ODmab-based linker of the invention, attachment of the primary sugar

residue and coupling of the sugar-linker conjugate to the resin support.

Figure 5 shows the cleavage of the oligosaccharide-linker bond in a resin-bound hydrazine mediated deprotection product.

Figure 6 shows a general representation of the selective deprotection of one sugar hydroxyl group, and subsequent coupling of the next sugar donor.

Figure 7 shows the immobilisation of an amino-oligosaccharide on the Dde-derivatised support.

Figure 8 shows a list of activated sugar donors for solid-phase synthesis.

Figure 9 shows the synthesis of a differentially protected thioglycoside and a partially protected aminoglycoside.

Figure 10 shows the trichloroacetimidate activation of the 4-aminobenzyl modified linker.

Figure 11 shows ammonia-mediated cleavage of the aminoglycoside with post-cleavage acidification to generate the free carbohydrate.

Figure 12 shows a specific example of the general strategy for oligosaccharide synthesis employing a thiogycoside as the sugar donor.

Figure 13 shows another specific example of the general strategy for oligosaccharide synthesis employing a thiogycoside as the sugar donor.

Figure 14 shows the cleavage of a monosaccharide bound to the 4-aminobenzyl modified linker.

Figure 15 shows an example of a resin-bound fully protected trisaccharide.

Figure 16 shows the immobilisation of an unprotected amino sugar.

Detailed Description of the Invention

Abbreviations used herein are as follows:

Bn	Benzyl
Bu	Butyl

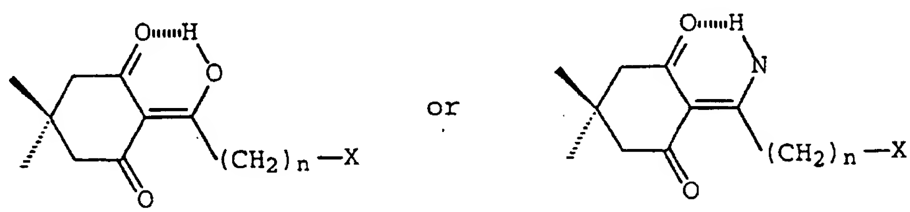
DCM	Dichloromethane
Dde	*N*-1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)ethyl
Ddh-OH	6-Hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)hexanoic acid
DMAP	*N,N'*-Dimethyl aminopyridine
DMF	*N,N'*-Dimethylformamide
DMTST	Dimethyl(methylthio)sulphonium trifluoromethanesulphonate
EEDQ	1-Isobutyloxycarbonyl-2-isobutyloxy-1,2-dihydroquinoline
EtOAc	Ethyl acetate
EtOH	Ethanol
FAB-MS	Fast atom bombardment mass spectrometry
HRMS	High resolution mass spectrometry
MBHA	Methyl benzyhydrylamine resin
Me	Methyl
MeOH	Methanol
NMR	Nuclear magnetic resonance
ODmab	4-{*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]-amino}benzyl alcohol.
PEG	Polyethylene glycol
tBu	tetra-butyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
TNBS	2,4,6-Trinitrobenzene sulphonic acid

The invention is based upon the immobilisation of a Dde-, Ddh or ODmab-based linker to a polymer support in order to tether any saccharide or oligosaccharide group. This has been illustrated by the coupling of *N*- and *O*-glycosides to the linkers, which have been used for oligosaccharide synthesis following coupling to the resin. The nature of these linkers is such that as well as the potential to immobilise any type of sugar, any sugar donor can be subsequently used for oligosaccharide synthesis, thereby allowing rapid and efficient coupling procedures.

Suitable sugar donors include, but are not limited to orthoesters, thioorthoesters, cyanoalkylidene derivatives, 1-*O*-acyl sugars, acetimidates, trichloroacetimidates, thioglycosides, glycosyl thiocyanates, pentenyl glycosides, pentenoylglycosides, isoprenyl glycosides, glycals, tetramethylphosphoro diamidates, sugar diazirines, selenoglycosides, phosphorodithioates, glycosyl-dialkylphosphites, glycosylsulphoxides and glycosylfluorides.

The stability of the linkers means that orthogonal hydroxy-protecting groups can be employed in sugar protection. These protecting groups include, but are not limited to, acyl-type, trityl, benzyl type, acetal type or various silyl and photolabile protecting groups.

The ease of linker synthesis means that the second functional group on the linker may be a halogen, alcohol, thiol or secondary amine, *eg*.



X = Halogen, OH, COOH, SH, NHR

Similarly, the ease of linker synthesis also means that any functionalised resin may be used to immobilise the linker, *eg*. MBHA resin, amino or carboxy tentagel resins, 4-sulfamylbenzoyl AM resin *etc*.

C-Acylation of dimedone with, for example, a mono-protected di-carboxylic acid is readily achieved *via* a carbodiimide activated, DMAP catalysed condensation in dry DCM. Removal of the ester protection and coupling of the first amino sugar residue generates a sugar-linker conjugate which can be coupled readily to an amino-functionalised resin support *via* a carbodiimide-mediated

condensation. This reaction can be monitored using conventional amine tests such as TNBS or ninhydrin, to ensure quantitative acylation. Alternatively, the linker can be synthesised directly on the resin, followed by introduction of the first sugar residue on to the linker-resin conjugate. Both methods are illustrated in Figure 3.

If an ether-type linkage between the primary sugar residue and the resin is required, then modification of the linker with 4-aminobenzylalcohol to generate the ODmab-type entity allows this method of chemical ligation, as illustated in Figure 4.

Following selective deprotection of one hydroxyl group, the second sugar residue is coupled using any of the sugar donors referred to above, as illustrated in Figure 8. A portion of the resin is readily cleaved using either ammonia, hydrazine or a primary amine, as shown in Figure 5, and the cleavage mixture is analysed by TLC to monitor the reaction progress. Completion of the reaction is indicated by the disappearance of the monosaccharide. The sequential deprotection and coupling of the following sugar residues is continued until the desired oligosaccharide is complete, as illustrated in Figure 1. The protecting groups are then removed, and the oligosaccharide is cleaved from the resin support using either ammonia, hydrazine, or a primary amine, in a suitable solvent.

The resin-linker system of the invention is ideal for the synthesis of combinatorial oligosaccharide libraries, as shown in Figure 2, and for the immobilisation of mono- or oligosaccharides, as shown in Figure 7.

The invention will now be described in detail by way of reference only to the following non-limiting examples.

Examples 1-5 Synthesis of a Specially Protected Thioglycoside-Type Sugar Donor (Figure 9)

1 *Ethyl 2,3,4,6-tetra-O-acetyl-1-thio-ß-D-galactopyranoside*

A mixture of galactose pentaacetate (38.00 g, 97.43 mmol), (ethylthio)trimethylsilane (19.60 g, 146.15 mmol) and trimethylsilyl trifluoromethanesulfonate (23.60 g, 106.20 mmol) in CH_2Cl_2 (150 ml) was stirred overnight at room temperature. The reaction mixture was diluted with CH_2Cl_2 (150 ml) and washed with 1M Na_2CO_3 solution (300 ml), water (300 ml), dried over $MgSO_4$ and concentrated. The residue was crystallised from hexane/di-isopropyl ether 1:1 (v/v) to give ethyl 2,3,4,6-tetra-O-acetyl-1-thio-ß-D-galactopyranoside (34.00 g, 89%).

R_f 0.43 (hexane/EtOAc 1:1); FAB MS $C_{16}H_{24}O_9S$ (392.3) m/z (%) 415 $[M+Na]^+$ (100), 393 $[M+H]^+$ (20), 331 (56).

2 *Ethyl 4,6-O-benzylidene-1-thio-β-D-galacto-pyranoside*

A mixture of ethyl 2,3,4,6-tetra-O-acetyl-1-thio-ß-D-galactopyranoside (10 g, 25.51 mmol) and sodium methoxide (200 mg, 3.7 mmol) was stirred in abs. MeOH (100 ml) at room temperature for 2 hours. The reaction mixture was neutralised with Amberlite IRA 120 (H+) ion exchange resin and evaporated. The residue was taken up in the (1:?) mixture of benzaldehyde/formic acid (21.2 ml) and stirred at room temperature for 90 minutes. The reaction mixture was diluted with ether (200 ml) and kept at -15°C for 2 hours. The precipitate formed was collected and purified by chromatography using $CHCl_3$/ethanol 10:3 (v/v) to give ethyl 4,6-O-benzylidene-1-thio-β-D-galacto-pyranoside (8.1 g, 64.5%).

R_f 0.64 ($CHCl_3$/ethanol 10:3).

3 *Ethyl 2,3-di-O-benzyl-4,6-O-benzylidene-β-D-galactopyranoside*

Ethyl 4,6-O-benzylidene-1-thio-β-D-galactopyranoside (6.90 g, 22.11 mmol) in 60 ml DMF was added dropwise at 0°C to a suspension of sodium hydride 60% (2.65 g, 66.34 mmol) in 60 ml DMF. The mixture was stirred at room temperature for 1 hour, then benzyl bromide (11.34 g, 66.34 mmol) was added dropwise at 0°C. The mixture was stirred at room temperature overnight. The mixture was evaporated, and xylene (2x50 ml) was distilled from the residue. The residue was taken up in ether (300 ml) and washed with 2x100 ml water. The organic layer was dried over $MgSO_4$, evaporated and crystallized from MeOH giving ethyl 2,3-di-O-benzyl-4,6-O-benzylidene-1-thio-β-D-galactopyranoside (8.90 g, 82%).

R_f 0.51 hexane/EtOAc 1:1 v/v); ^1H NMR ($CDCl_3$) δ 7.55-7.25 (m, 15H, 15 Ar-H), 5.47 (s, 1H, CHAr), 4.88-4.75 (4d, 4H, 2 CH_2Ar), 4.44 (d, 1H, H-1, $J_{1,2}$=10.89 Hz), 4.30 (dd, 1H, H-6'), 4.16 (d, 1H, H-4), (3.97 (dd, 1H, H-3), 3.88 (t, 1H, H-2), 3.60 (dd, 1H, H-6), 3.35 (d, 1H, H-5), 2.90-2.40 (m, 2H, CH_2S), 1.33 (t, 3H, Me); FAB MS $C_{29}H_{32}O_5S$ (492.40) m/z (%) 515 $[M+Na]^+$ (100), 493 $[M+H]^+$ (41), 431 (53).

4 *Ethyl 2,3,6-tri-O-benzyl-1-thio-β-D-galacto-pyranoside*

A mixture of crude ethyl 2,3-di-O-benzyl-4,6-O-benzylidene-1-thio-β-D-galactopyranoside (5.4 g, 10.97 mmol), sodium cyanoborohydride (6.89 g, 109.7 mmol) and a few grains of methyl orange indicator was stirred in THF (60 ml) at 0°C. THF saturated with HCl was added very slowly until a permanent pink colour was obtained. The reaction mixture was stirred at room temperature for 20 min, then neutralised with dry NH_3 and evaporated. The residue was taken up in $CHCl_3$ (100 ml), washed with saturated $NaHCO_3$ solution (50 ml), dried over $MgSO_4$ and evaporated. The residue was dissolved in MeOH (50 ml), reflux for 10 min and evaporated. The crude product was

purified by chromatography using 1,2-dichloroethane/ethyl acetate 10:0.5 as the mobile phase to give methyl 2,3,6-tri-O-benzyl-1-thio-β-D-galactopyranoside (4.14 g, 75%).

R_f 0.43 (1,2-dichloroethane/EtOAc 10:0.5 v/v); ^{1}H NMR ($CDCl_3$) δ 7.40-7.26 (m, 15H, 15 Ar-H), 4.88, 4.76,4.73, 4.71 (4d, 4H, 2 CH_2Ar), 4.57 (s, 2H, CH_2Ar), 4.42 (d.1H, H-1, $J_{1,2}$=9.64 Hz), 4.10 (m, 1H, H-4), (3.76 (dd, 1H, H-3), 3.67 (t, 1H, H-2), 3.55 (m, 2H, H-6), 2.75 (m, 2H, CH_2S), 2.50 (bs, 1H, OH), 1.31 (t, 3H, CH_3); FAB MS $C_{29}H_{34}O_5S$ (494.61) m/z (%) 627 $[M+Cs]^+$ (70), 517 $[M+Na]^+$ (30), 495 $[M+H]^+$ (12).

5 *Ethyl 2,3,6-tri-O-benzyl-4-bromoacetyl-1-thio-β-D-galactopyranoside*

A mixture of ethyl 2,3,6-tri-O-benzyl-1-thio-β-D-galactopyranoside (4.14 g, 8.38 mmol), sym. collidine (3.65 g, 30.16 mmol), and 4-dimethylaminopyridine in dry CH_2Cl_2 (60 ml) was stirred at 0°C and bromoacetyl bromide (2.53, 2.57 mmol) in CH_2Cl_2 added dropwise in 15 minutes. The reaction mixture was diluted with CH_2Cl_2 (100 ml) and washed with 5% HCl solution (3x30 ml) and saturated $NaHCO_3$ solution (30 ml). The solution was dried over $MgSO_4$ and evaporated. The residue was purified by chromatography using hexane/EtOAc 2:1 as the mobile phase to give ethyl 2,3,6-tri-O-benzyl-4-bromoacetyl-1-thio-β-D-galacto-pyranoside (4.84 g, 94%)

^{1}H NMR ($CDCl_3$) δ 7.40-7.25 (m, 15H, 15 Ar-H), 4.80-4.50 (m, 6H, 3 CH_2Ar), 4.45 (d, 1H, H-1, $J_{1,2}$=9.53 Hz), 2.73 (m, 2H, CH_2S), 1.30 (t, 3H, CH_3); FAB MS $C_{31}H_{35}BrO_6S$ (615.56) m/z (%) 638 $[M+Na]^+$ (15), 616 $[M+H]^+$ (32), 509 (80), 463 (21), 419 (18).

Examples 6-10 Synthesis of a Partially-Protected Glycosyl Amine (Figure 9)

6 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl azide

1,2,3,4,6-penta-O-acetyl-galactopyranose (1.17 g, 3 mmol) was dissolved in dry CH_2Cl_2 (15 ml), then trimethylsilyl azide (416 mg, 3.6 mmol) and $SnCl_4$ (0.18 ml) were added under nitrogen. The mixture was stirred at room temperature for 24 hours. The reaction mixture was subsequently diluted with CH_2Cl_2 (40 ml), dried over $MgSO_4$ and evaporated. The residue was purified by chromatography using hexane/EtOAc 8:7 v/v as the mobile phase to give 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl azide (1.05 g, 94%).

R_f 0.74 (hexane/EtOAc 8:7 v/v); ^{1}H NMR ($CDCl_3$) δ 5.41 (d, 1H, H-4), 5.17 (t, 1H, H-2), 5.04 (dd, 1H, H-3), 4.60 (d,1H, H-1, $J_{1,2}$=10.09 Hz), 4.19 (m, 2H, H-6), 4.00 (m, 1H, H-5), 2.15-1.98 (4s, 12H, 4 OAc); FAB MS $C_{14}H_{19}N_3O_9$ (373.32) m/z (%) 396 $[M+Na]^+$ (100), 374 $[M+H]^+$ (35), 331 (23).

7 4,6-O-benzylidene-β-D-galactopyranosyl azide

A mixture of 2,3,4,6-tetra-O-acetyl-ß-D-galacto-pyranosyl azide (19.35 g, 51.79 mmol) and sodium methoxide (200 mg, 3.7 mmol) was stirred in abs. MeOH (100 ml) at room temperature for 2 hours. The reaction mixture was neutralised with Amberlite IRA 120 (H+) ion exchange resin and evaporated. The residue was taken up in the mixture of benzaldehyde/formic acid (1:1) (52 ml) and stirred at room temperature for 90 minutes. The reaction mixture was evaporated and the residue was taken up in ether (60 ml) and kept at -15°C for 2 hours. The precipitate formed was collected by filtration and dried at room temperature affording 4,6-O-benzylidene-β-D-galactopyranosyl azide (11.8 g 78%).

R_f 0.64 ($CHCl_3$/ethanol 10:1.5).

8 2,3-di-O-benzyl-4,6-O-benzylidene-β-D-galacto-pyranosyl azide

4,6-O-benzylidene-β-D-galactopyranosyl azide (11.8 g, 40.27 mmol) in 60 ml DMF was added dropwise at 0°C to a suspension of sodium hydride 60% (6.21 g, 155.38 mmol) in 60 ml DMF. The mixture was stirred at room temperature for 1 hour, then benzyl bromide (26.57 g, 155.38 mmol) was added dropwise at 0°C. The mixture was stirred at room temperature overnight. The mixture was evaporated, and xylene (2x50 ml) was distilled from the residue. The residue was taken up in ether (500 ml) and washed with 2x100 ml water. The organic layer was dried over $MgSO_4$ and evaporated, giving methyl 2,3-di-O-benzyl-4,6-O-benzylidene-β-D-galactopyranosyl azide as a crude residue (19.4 g).

9 2,3,6-tri-O-benzyl-β-D-galactopyranosyl azide

A mixture of crude 2,3-di-O-benzyl-4,6-O-benzylidene-β-D-galactopyranosyl azide (9.00 g, 19.02 mmol), sodium cyanoborohydride (12.00 g, 190.2 mmol) and a few grains of methyl orange indicator was stirred in THF (80 ml) at 0°C. THF saturated with HCl was added very slowly until a permanent pink colour was obtained. The reaction mixture was stirred at room temperature for 20 min, then neutralised with dry NH_3 and evaporated. The residue was taken up in $CHCl_3$ (100 ml), washed with saturated $NaHCO_3$ solution (50 ml), dried over $MgSO_4$ and evaporated. The residue was dissolved in MeOH (50 ml) and kept under reflux for 10 min and evaporated. The crude product was purified by chromatography using 1,2-dichloroethane/EtOAc 10:0.4 as the mobile phase to give 2,3,6-tri-O-benzyl-β-D-galactopyranosyl azide (6.50 g, 72%).

R_f 0.42 (1,2-dichloroethane/EtOAc 10:0.4 v/v); ^1H NMR ($CDCl_3$) δ 7.40 (m, 15H, 15 Ar-H), 4.90-4.55 (m, 6H, 3 CH_2Ar), 4.06 (m, 1H, H-4), (3.82-3.70 (m, 3H, H-3, H-2, H-5), 3.65 (dd, 1H, H-6'), 3.60 (d, 1H, H-1, $J_{1,2}$= 8.64 Hz),

3.51 (dd, 1-H, H-6); FAB MS $C_{27}H_{29}N_3O_5$ (475.40) m/z (%) 608 $[M+Cs]^+$ (10), 498 $[M+Na]^+$ (65), 476 $[M+H]^+$ (25), 433 (75), 341 (20).

10 2,3,6-tri-O-benzyl-β-D-galactopyranosyl amine

A mixture of 2,3,6-tri-O-benzyl-β-D-galactopyranosyl azide (3.00 g, 6.31 mmol), propane-1,3-dithiol (3.40 g, 31.50 mmol), and triethylamine (3.50 g, 31.5 mmol) in MeOH (31 ml) was stirred under nitrogen at room temperature for 10 hours. The reaction mixture was evaporated and purified by chromatography using $CHCl_3$/EtOH 10:0.3 v/v to give 2,3,6-tri-O-benzyl-β-D-galactopyranosyl amine (2.66 g, 94%);

R_f 0.38 ($CHCl_3$/EtOH 10:0.3 v/v); FAB MS $C_{27}H_{31}NO_5$ (449.33) m/z (%) 472 $[M+Na]^+$ (75), 450 $[M+H]^+$ (100).

Example 11 Synthesis of a Glycosyl Amine - Ddh-Benzyl Ester Conjugate in Solution (Figure 3)

11 N-(Benzyl 6-(4,4-dimethyl-2,6-dioxocyclo-hexylidene)-hexanoate-6-yl) 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl amine

A mixture of benzyl 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate (932 mg, 2.60 mmol), 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl amine in CH_2Cl_2 (2.0 ml) was stirred at room temperature for 2 days. The reaction mixture was evaporated and purified by chromatography using hexane/EtOAc 1:1 as the mobile phase to give N-(Benzyl 6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate-6-yl) 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl amine (1.70 g, 95%);

R_f 0.32 (hexane/EtOAc 1:1 v/v); ^1H NMR ($CDCl_3$) δ 7.37-7.26 (m, 5H, 5 Ar-H), 5.40-5.00 (m, 7H, 7 sugar protons), 3.10, 2.85 (2t, 4H, 2 CH_2), 2.38 (2s, 4H, Dde 2 CH_2), 2.06-1.98 (4s, 12H, 4 OAc), 1.80 (m, 4H, 2 CH_2), 1.02, 1.00 (2s, 6H,

Dde $2CH_3$); FAB MS $C_{35}H_{45}NO_{13}$ (687.23) m/z (%) 710 $[M+Na]^+$ (35), 688 $[M+H]^+$ (100), 356 (60).

Example 12 Synthesis of a Fully Protected Glycosyl Amine - Ddh Conjugate Deprotecting a "Fully Protected Amine - DdH Ester Conjugate" in Solution (Figure 3)

12 N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl amine

N-(Benzyl 6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate-6-yl) 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl amine (1.27 g, 1.84 mmol) was hydrogenated over Pd/C (10%) (200 mg) in MeOH (20 ml) at room temperature for 10 hours. The catalyst was filtered off, and the filtrate was evaporated and then chromatographed using $CHCl_3$/MeOH 10:0.5 v/v as the mobile phase to give N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl amine 1.10 g, 98%);

R_f 0.38 ($CHCl_3$/MeOH 10:0.5 v/v); ^1H NMR ($CDCl_3$) δ 5.40-5.00 (m, 7H, 7 sugar protons), 3.15, 2.86 (2t, 4H, 2 CH_2), 2.45 (2s, 4H, Dde 2 CH_2), 2.10-1.98 (4s, 12H, 4 OAc), 1.80-1.65 (m, 4H, 2 CH_2), 1.02, 1.00 (2s, 6H, Dde $2CH_3$); FAB MS $C_{28}H_{39}NO_{13}$ (597.33) m/z (%) 620 $[M+Na]^+$ (55), 598 $[M+H]^+$ (100).

Example 13 Synthesis of a Glycosyl Amine - Ddh-Methyl Ester Conjugate in Solution (Figure 3)

13 N-(Methyl 6-(4,4-dimethyl-2,6-dioxocyclo-hexylidene)-hexanoate-6-yl) 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl amine

Reaction 11 was repeated with the difference that methyl 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate was used instead of benzyl 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate. Yield: 92%;

R_f 0.28 (hexane/EtOAc 1:1 v/v);FAB MS $C_{29}H_{41}NO_{13}$ (611.45) m/z (%) 624 $[M+Na]^+$ (100), 612 $[M+H]^+$ (34).

Example 14 Synthesis of a Glycosyl Amine - Ddh-t-Butyl Ester Conjugate in Solution (Figure 3)

14 N-(t-Butyl 6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate-6-yl) 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl amine

Reaction 11 was repeated with the difference that t-butyl 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate was used instead of benzyl 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate. Yield: 96%;

R_f 0.35 (hexane/EtOAc 1:1 v/v); FAB MS $C_{32}H_{47}NO_{13}$ (653.37) m/z (%) 676 $[M+Na]^+$ (80), 677 $[M+H]^+$ (100).

Example 15 Synthesis of Ddh-OH Benzyl Ester in Solution (Figure 3)

*15 Benzyl 6-hydroxy-6-(4,4-dimethyl-2,6-dioxo-*cyclohexylidene)-hexanoate

To a stirred solution of mono-benzyl adipate (2.36g, 10 mmol) in dry CH_2Cl_2 (50 ml) was added 5,5-dimethyl-1,3-cyclohexanedione (1.4 g, 10 mmol), N,N'-dicyclohexylcarbodiimide (2.1 g, 10.1 mmol) and 4-dimethylaminopyridine (1.22 g, 10 mmol). The resulting solution was allowed to stir at room temperature for 18 h. The solution was cooled and filtered to remove the precipitated N,N'-dicyclohexylurea. The filtrate was evaporated and the residue redissolved in EtOAc (50 ml) and washed with 1 M $KHSO_4$. The organic extract was washed with brine (92x10 ml), dried ($MgSO_4$) and evaporated to yield a white/yellow amorphous powder. Flash silica chromatography (EtOAc/hexane 1:2 v/v) afforded benzyl 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate (3.00 g, 84%) as a white crystalline solid.

^1H NMR ($CDCl_3$) δ 18.10 (s, 1H, OH), 7.30 (s, 5H, 5Ar-H), 5.06 (s, 2H, CH_2Ar), 3.00 (t, 2H, CH_2), 2.47 (s, 2H, Dde CH_2), 2.35 (t, 2H, CH_2CO_2), 2.29 (s, 2H, Dde CH_2), 1.65 (m, 4H, 2 CH_2), 1.01 (s, 6H, 2 CH_3); FAB MS $C_{21}H_{26}O_5$ (358.18) m/z (%) 359 $[M+H]^+$ (100), 267 (40); HRMS (FAB) Found: m/z 359.1858 Calcd for $C_{21}H_{27}O_5$:
(M+H), 359.1850.

<u>Example 16</u> <u>Synthesis of Ddh-OH by Deprotection of a Ddh-OH Ester</u> (Figure 3)

16 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclo-hexylidene)-hexanoic acid

Benzyl 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclo-hexylidene)-hexanoate (1.50 g, 4.19 mmol) was hydrogenated over Pd/C (10 %) (150 mg) in MeOH (20 ml) at room temperature for 10 hours. The catalyst was filtered off, and the filtrate was evaporated, yielding 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid (1.10 g, 98%);

R_f 0.35 (hexane/EtOAc 2:1 v/v); FAB MS $C_{14}H_{20}O_5$ (268.12) m/z (%) 313 $[M+2Na]^+$ (34), 291 $[M+Na]^+$ (100), 269 $[M+H]^+$ (16).

<u>Example 17</u> <u>Synthesis of a Ddh-OH Methyl Ester in Solution</u> (Figure 3)

17 Methyl 6-hydroxy-6-(4,4-dimethyl-2,6-dioxo-cyclohexylidene)-hexanoate

Reaction 15 was repeated, with the difference that mono-methyl adipate was used instead of mono-benzyl adipate, and afforded methyl 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate (2.39 g, 85%).

R_f 0.32 (EtOAc/hexane 1:2 v/v) FAB MS $C_{15}H_{22}O_5$ (282.22) m/z (%) 305 $[M+H]^+$ (100), 283 $[M+H]^+$ (66).

Example 18 Synthesis of Ddh-OH t-Butyl Ester in Solution (Figure 3)

18 t-Butyl 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclo-hexylidene)-hexanoate

Reaction 15 was repeated, with the difference that mono-t-butyl adipate was used instead of mono-benzyl adipate, and afforded t-butyl 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate (2.62 g, 81%).

R_f 0.36 (EtOAc/hexane 1:2 v/v) FAB MS $C_{18}H_{28}O_5$ (324.41) m/z (%) 347 $[M+H]^+$ (100), 325 $[M+H]^+$ (43), 267 (80).

Example 19 Synthesis of Ddh-OH by Deprotection of a Ddh-OH t-Butyl Ester (see 16, Figure 3)

19 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclo-hexylidene)-hexanoic acid

t-Butyl 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclo-hexylidene)-hexanoate (100 mg, 0.30 mmol) was dissolved in CH_2Cl_2/TFA 1:1 mixture (2 ml) and stirred at room temperature for 1 h. The reaction mixture was evaporated giving 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid (0.81 g, 98%)

Example 20 Synthesis of Ddh-OH from Cyclic Anhydrides (see 16, Figure 3)

20 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclo-hexylidene)-hexanoic acid

A mixture of glutaric anhydride (2.28 g, 20 mmol), dimedone (2.8 g, 20 mmol), 4-dimethylamino-pyridine (3.99 g, 30 mmol) in abs. pyridine (50 ml) was stirred at room temperature for 24 h. The reaction mixture was evaporated and the residue was taken up in $CHCl_3$ (100 ml), washed 5% HCl solution (3x25 ml), saturated $NaHCO_3$ solution, dried over $MgSO_4$ and evaporated. The residue was purified by chromatography using ether/acetic acid (10 ml:1 drop) as the mobile phase to give 6-hydroxy-

6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid (2.28 g, 45%).

Example 21 Synthesis of a Fully Protected Glycosyl Amine - Ddh Conjugate Using Ddh-OH in Solution (See 12, Figure 3)

21 *N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl amine*

A mixture of 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid (400 mg, 1.49 mmol), 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl amine (259 mg, 0.74 mmol) in abs. EtOH was stirred under reflux for 2 h. The reaction mixture was evaporated and chromatographed using $CHCl_3$/MeOH 10:0.5 v/v to give N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl amine(410 mg, 92%).

Example 22 Synthesis of a Partially Protected Glycosyl Amine - Ddh Conjugate Using Ddh-OH in Solution (Figure 3)

22 *N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 2,3,6-tri-O-benzyl-β-D-galactopyranosyl amine*

Reaction 21 was repeated with the difference that 2,3,6-tri-O-benzyl-β-D-galactopyranosyl amine was used instead of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl amine, and afforded N-(6-(4,4-dimethyl-2,6-dioxocyclo-hexylidene)-hexanoic acid-6-yl) 2,3,6-tri-O-benzyl-β-D-galactopyranosyl amine (299 mg, 90%).

R_f 0.34 ($CHCl_3$/MeOH 10:0.1 v/v) FAB MS $C_{37}H_{43}NO_7$ (613.41) m/z (%) 649 $[M+2Na]^+$(34), 626 $[M+Na]^+$ (100), 614 $[M+H]^+$ (65).

Example 23 Synthesis of Ddh-Aminobenzyl Linker in Solution (Figure 4)

23 *N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 4-amino-benzylalcohol*

Reaction 21 was repeated with the difference that 4-aminobenzyl alcohol was used instead of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl amine, and afforded N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 4-aminobenzyl alcohol (259 mg, 94%).

R_f 0.40 (EtOAc/hexane/acetic acid 2:1:0.1 v/v/v); FAB MS $C_{21}H_{27}NO_5$ (373.43) m/z (%) 418 $[M+2Na]^+$(24), 396 $[M+Na]^+$ (100), 374 $[M+H]^+$ (35).

Example 24 Synthesis of Ddh-Aminobenzyl t-Butyl Ester Linker in Solution (Figure 4)

24 *N-(t-Butyl 6-(4,4-dimethyl-2,6-dioxocyclo-hexylidene)*-hexanoate-6-yl) 4-aminobenzyl alcohol

A mixture of t-butyl 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate (400 mg, 1.23 mmol) and 4-aminobenzyl alcohol (605 mg, 4.92 mmol) in abs. EtOH was stirred under reflux for 2 h. The reaction mixture was evaporated and purified by chromatography using $CHCl_3$/MeOH 9:1 as the mobile phase to give N-(t-Butyl 6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate-6-yl) 4-aminobenzyl alcohol (395 mg, 75%)

R_f 0.52 ($CHCl_3$/MeOH 9:1 v/v) FAB MS $C_{25}H_{35}NO_5$ (429.53) m/z (%) 452 $[M+Na]^+$ (100), 430 $[M+H]^+$ (32), 372 (64).

Example 25 Synthesis of Ddh-Aminobenzyl t-Butyl Ester Trichloroacetimidate Activated Linker in Solution (Figure 4)

25 *N-(t-Butyl 6-(4,4-dimethyl-2,6-dioxocyclohexylidene)*-hexanoate-6-yl) 4-aminobenzyl trichloroacetimidate

A mixture of N-(t-butyl 6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate-6-yl) 4-aminobenzyl alcohol (500 mg, 1.16 mmol) and trichloroacetonitrile (503 mg, 3.49 mmol) in CH_2Cl_2 (5 ml) was stirred at 0°C and 1,8-diazabicyclo(5.4.0)undec-7-ene (5 mg, 0.03 mmol) added. The reaction mixture was stirred at 0°C for 90 minutes, at room temperature for 2 h, then evaporated. The residue was purified by chromatography using EtOAc/hexane 1:1 as the mobile phase to give N-(t-butyl 6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate-6-yl) 4-aminobenzyl trichloroacetimidate (580 mg, 87%);

R_f 0.41 (EtOAc/hexane 1:1 v/v); FAB MS $C_{27}H_{35}Cl_3N_2O_5$ (573.94) m/z (%) 595 $[M+Na]^+$ (100), 753 $[M+H]^+$ (40), 515 (39), 430 (54).

Example 26 Synthesis of a Fully Protected Sugar (Sugar-Linker Bond is not at the Glycosidic Position) - Ddh-Aminobenzyl t-Butyl Ester Conjugate Via Trichloroacetimidate Activation (Figure 4)

26 *Benzyl 2-acetamido-3-O-acetyl-6-O-benzyl-2-deoxy-4-O-[N-(t-butyl 6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate-6-yl) 4-aminobenzyl]-α-D-glucopyranoside*

N-(t-Butyl 6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate-6-yl) 4-aminobenzyl trichloroacetimidate (400 mg, 0.70 mmol) was added at 20°C under nitrogen to a solution of Benzyl 2-acetamido-3-O-acetyl-6-O-benzyl-2-deoxy-α-D-glucopyranoside (155 mg, 0.35 mmol) in CH_2Cl_2 (6 ml). Trifluoromethanesulphonic acid in ether

(0.1 M, 0.06 ml) was added and the mixture was stirred for 30 min at 20°C. The reaction was stopped with 5% $NaHCO_3$ solution (0.25 ml).After filtration of the mixture and evaporation of the filtrate, the crude residue was purified by chromatography using EtOAc/hexane 2:1 v/v as the mobile phase to give Benzyl 2-acetamido-3-O-acetyl-6-O-benzyl-2-deoxy-4-O-[N-(t-butyl 6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate-6-yl) 4-aminobenzyl]-α-D-glucopyranoside (210 mg, 71%).

R_f 0.37 (EtOAc/hexane 2:1 v/v); FAB MS $C_{49}H_{62}N_2O_{11}$ (855.01) m/z (%) 877 $[M+Na]^+$ (100), 855 $[M+H]^+$ (35), 797 (73).

Example 27 Synthesis of a Fully Protected Glycoside (Sugar-Linker Bond at the Glycosidic Position) - Ddh-Aminobenzyl Linker - Resin Via Trichloroacetimidate Activation (Figure 4)

27 [N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 4-aminobenzyl] 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside MBHA resin conjugate

N-(t-Butyl 6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate-6-yl) 4-aminobenzyl trichloroacetimidate (400 mg, 0.70 mmol) was added at 20°C under nitrogen to a solution of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranose (121 mg, 0.35 mmol) in CH_2Cl_2 (6 ml). Trifluoromethanesulphonic acid in ether (0.1 M, 0.06 ml) was added and the mixture was stirred for 30 min at 20°C. The reaction was stopped with 5% $NaHCO_3$ solution (0.25 ml). After filtration of the mixture, the filtrate was evaporated. The unpurified residue was taken up in CH_2Cl_2/TFA mixture (1:1) (5 ml), stirred at room temperature for 1 h and evaporated. The resulting acid was dissolved in CH_2Cl_2 (5 ml), N,N'-diisopropylcarbodiimide (128 mg, 1 mmol) added, and the mixture was gently agitated with MBHA resin (100 mg)(swelled in DMF for 20 min.) for 30 min.

Example 28 Synthesis of a Fully Protected Glycoside (Sugar - Linker Bond is at the Glycoside Position) - Ddh-Aminobenzyl Benzyl Ester Conjugate Via DMTST Promoted Glycosylation (see 26, Figure 4)

28 [N-[Benzyl (6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate]-6-yl 4-aminobenzyl]-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside

A mixture of N-[Benzyl (6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate]-6-yl 4-aminobenzyl alcohol (500 mg, 1.08 mmol), methyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (400 mg, 1.08 mmol) in CH_2Cl_2 (10 ml) was stirred at room temperature and DMTST (835 mg, 3.24 mmol) added. The solution was stirred at room temperature for 1 h and washed with saturated $NaHCO_3$ solution (3 ml), dried over $MgSO_4$ and evaporated. The residue was purified by chromatography using hexane/EtOAc 1:1 v/v as the mobile phase to give [N-[Benzyl (6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate]-6-yl 4-aminobenzyl]-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (610 mg, 75%).

R_f 0.47 (hexane/EtOAc 1:1 v/v); FAB MS $C_{42}H_{51}NO_{14}$ (793.83) m/z (%) 816 $[M+Na]^+$ (100), 794 $[M+H]^+$ (25), 702 (66).

Example 29 Synthesis of a Fully Protected Glycoside (Sugar-Linker Bond is at the Glycosidic Position) - Ddh-Aminobenzyl Linker - Resin Conjugate Via DIPCDI Activation (see 27, Figure 4)

29 [N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 4-aminobenzyl]-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside MBHA resin conjugate

[N-[Benzyl (6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate]-6-yl 4-aminobenzyl]-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (500 mg, 0.63 mmol) was

hydrogenated over Pd/C (10%) (200 mg) in MeOH (20 ml) at room temperature for 10 hours. The catalyst was filtered off and the filtrate was evaporated. The residue was dissolved in CH_2Cl_2 (5 ml), N,N'-diisopropylcarbodiimide (128 mg, 1 mmol) added, and the mixture was gently agitated with MBHA resin (200 mg) (pre-swelled in DMF for 20 min.) for 30 min.

<u>Example 30</u> <u>Synthesis of a Partially Protected Glycosyl Amine - Ddh Conjugate Using Ddh-OH t-Butyl Ester in Solution</u> (see 22, Figure 3)

30 N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 2,3,6-tri-O-benzyl-β-D-galactopyranosyl amine

A mixture of t-butyl 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate (400 mg, 1.23 mmol) and 2,3,6-tri-O-benzyl-β-D-galactopyranosyl amine (276 mg, 0.61 mmol) in abs. EtOH (10 ml) was stirred under reflux for 2 h. The reaction mixture was evaporated. The residue was taken up in CH_2Cl_2/TFA mixture (1:1) (10 ml) and stirred at room temperature for 1 h. The reaction mixture was evaporated and purified by chromatography using $CHCl_3$/MeOH 10:0.1 v/v as the mobile phase to give N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 2,3,6-tri-O-benzyl-β-D-galactopyranosyl amine (280 mg, 73%).

R_f 0.34 ($CHCl_3$/MeOH 10:0.1 v/v) FAB MS $C_{37}H_{43}NO_7$ (613.41) m/z (%) 649 $[M+2Na]^+$(34), 626 $[M+Na]^+$ (100), 614 $[M+H]^+$ (65).

Example 31 Synthesis of a Fully Protected Glycosyl Amine - Ddh - Resin Conjugate Where the Resin Coupling is the Final Step (Figure 3)

31 N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl amine - MBHA conjugate

MBHA resin (Subst. ratio: 0.42 mmol/g) (200 mg) bearing a total amine functionality of 0.084 mmol was swollen in DMF for 20 min. The resin was then washed with fresh DMF and N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 2,3,4,6-tetra-O-acetyl-β-D-gluco-pyranosyl amine (200 mg, 4 equiv.) and N,N'-diisopropyl-carbodiimide (53 µl,4 equiv.) were added in DMF (5 ml) and the resin gently agitated for 30 min. The TNBS test was faintly positive so using the above conditions, a double coupling was performed, this time producing a negative TNBS test result. The resin was washed with DMF, methanol and finally ether. The resin was then allowed to dry in vacuum over KOH overnight.

Example 32 Synthesis of a Fully Protected Sugar (Sugar - Linker Bond is Not at the Glycosidic Position) - Ddh - Resin Conjugate Where the Resin Coupling is the Final Step (see 27, Figure 4)

32 Benzyl 2-acetamido-3-O-acetyl-6-O-benzyl-2-deoxy-4-O-[N-(6-(4,4-dimethyl-2,6-dioxocyclohexyl-idene)-hexanoic acid-6-yl) 4-aminobenzyl]-α-D-glucopyranoside - MBHA resin conjugate

Benzyl 2-acetamido-3-O-acetyl-6-O-benzyl-2-deoxy-4-O-[N-(t-butyl 6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate-6-yl) 4-aminobenzyl]-α-D-glucopyranoside (290 mg, 0.33 mmol) was dissolved in CH_2Cl_2/TFA mixture (1:1) and stirred at room temperature for 1 h. The reaction mixture was evaporated, and procedure 31 was used to bind the compound to the MBHA resin.

Example 33 Synthesis of Ddh-Aminobenzyl Linker - Resin Conjugate With Selective Resin Coupling (Unprotected Hydroxyl Group is Present on the Linker) (Figure 10)

33 *N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 4-amino-benzylalcohol - MBHA resin conjugate*

MBHA resin (100 mg) bearing a total amine functionality of 0.042 mmol was swelled in DMF for 20 min. The resin was then washed with fresh DMF and N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 4-aminobenzyl alcohol (63 mg, 4 equiv.) and 1-isobutyloxy-carbonyl-2-isobutyloxy-1,2-dihydroquinoline (EEDQ) (51 mg, 4 equiv.) were added in DMF (5 ml) and the resin gently agitated for 24 h. The TNBS test was faintly positive so using the above conditions, a double coupling was performed, this time producing a negative TNBS test result. The resin was washed with DMF (5x10 ml).

Example 34 Synthesis of Ddh-Aminobenzyl Trichloroacetimidate Activated Linker - Resin Conjugate When the Activation Takes Place on the Resin (Figure 10)

34 *N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoate-6-yl) 4-aminobenzyl trichloroacetimidate - MBHA resin conjugate*

Resin from Example 33 was treated with trichloroacetonitrile (50 mg, 0.33 mmol) in CH_2Cl_2 (1 ml) was stirred at 0°C and 1,8-diazabicyclo(5.4.0)undec-7-ene (1 mg, 0.003 mmol) added. The reaction mixture was stirred at 0°C for 90 minutes, at room temperature for 2 h, then the resin was filtered off and washed with DMF (5x10 ml).

Example 35 Synthesis of a Fully Protected Sugar (Sugar - Linker Bond is Not at the Glycosidic Position) - Ddh - Resin Conjugate When the Sugar Coupling is the Final Step (see 32, Figure 4)

35 *Benzyl 2-acetamido-3-O-acetyl-6-O-benzyl-2-deoxy-4-O-[N-(6-(4,4-dimethyl-2,6-dioxocyclo-hexylidene)-hexanoic acid-6-yl) 4-aminobenzyl]-α-D-glucopyranoside - MBHA resin conjugate*

Resin from Example 34 was added at room temperature to a solution of Benzyl 2-acetamido-3-O-acetyl-6-O-benzyl-2-deoxy-α-D-glucopyranoside (75 mg, 0.16 mmol) in CH_2Cl_2 (1 ml). Trifluoromethanesulphonic acid in ether (0.1 M, 60 µl) was added and the mixture was stirred for 30 min. The reaction was stopped with triethylamine (120 µl) and washed with DMF (5x10 ml).

Example 36 First Step of the Solid Phase Synthesis of the Resin - Ddh- or DdH-Aminobenzyl - Linker (Figure 3)

36 *Adipic acid - MBHA resin conjugate*

MBHA resin (1.0 g) bearing a total amine functionality of 0.42 mmol was swelled in DMF for 20 min. The resin was then treated with a mixture of adipic acid (1.41 g, 10 mmol) and N,N'-diisopropylcarbodiimide in CH_2Cl_2 (10 ml) for 60 min. A second coupling was performed in DMF to get a negative ninhydrin test. The resin was washed with DMF (5x10 ml).

Example 37 Second Step of the Solid Phase Synthesis of the Resin - Ddh- or DdH-Aminobenzyl - Linker (Figure 3)

37 *6-Hydroxy-6-(4,4-dimethyl-2,6-dioxocyclo-hexylidene)-hexanoic acid - MBHA resin conjugate*

To the resin from Example 36 a mixture of 5,5-dimethyl-1,3-cyclohexanedione (280 mg, 2.0 mmol), N,N'-dicyclohexylcarbodiimide (283 mg, 2.00 mmol) and

4-dimethylaminopyridine (244 mg, 2.00 mmol) was added in CH_2Cl_2 (10 ml) and stirred at room temperature for 18 h. The resin was washed with DMF (5x10 ml).

Example 38 Solid Phase Synthesis of a Fully Protected Glycosyl Amine - Ddh - Resin Conjugate (see 31, Figure 3)

38 N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl amine - MBHA resin conjugate

The resin from Example 37 was reacted with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl amine (712 mg, 2.00 mmol) in DMF (5 ml) at room temperature for 2 days. The resin was washed with DMF (5x10 ml).

Example 39 Solid Phase Synthesis of a Partially Protected Glycosyl Amine - Ddh - Resin Conjugate (Figure 3)

39 N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 2,3,6-tri-O-benzyl-β-D-galactopyranosyl amine - MBHA resin conjugate

The resin from Example 37 was reacted with 2,3,6-tri-O-benzyl-β-D-galactopyranosyl amine (900 mg, 2.00 mmol) in abs. EtOH under reflux for 2 h. The resin was washed with DMF (5x10 ml).

Example 40 Solid Phase Synthesis of Ddh-Aminobenzyl Linker - Resin Conjugate (see 33, Figure 10)

40 N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 4-amino-benzylalcohol - MBHA resin conjugate

A mixture of resin from Example 37 and 4-aminobenzyl alcohol (246 mg, 2.00 mmol) in abs. EtOH was stirred under reflux for 2 h, then washed with DMF (5x10 ml).

Example 41 Cleavage of a Fully Protected Glycosyl Amine - Ddh - Resin Conjugate Affording Fully Protected Glycosyl Amine (Figure 11)

41 *Cleavage of N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl amine - MBHA resin conjugate by NH_3 treatment.*

Resin from Example 38 (10 mg) was treated with saturated NH_3/MeOH solution (0.2 ml) at room temperature for 5 min. The resin was filtered off, the filtrate was evaporated, giving 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl amine in quantitative yield.

Example 42 Cleavage of a Fully Protected Glycosyl Amine - Ddh - Resin Conjugate Affording Fully Protected Reducing Sugar

42 *Cleavage of N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl amine - MBHA resin conjugate by NH_3 treatment, affording a reducing carbohydrate derivative (Figure 11).*

Resin from Example 38 (10 mg) was treated with saturated NH_3/MeOH solution (0.2 ml) at room temperature for 5 min. The resin was filtered off, the filtrate was evaporated. The residue was dissolved in the mixture of acetone/water 10:1 v/v (0.2 ml), acidified with acetic acid (20 μl) and stirred at room temperature for 1 h. The solution was evaporated giving 2,3,4,6-tetra-O-acetyl-β-D-glucopyranose in quantitative yield.

Example 43 Carbohydrate Deprotection of the Fully Protected Sugar -Ddh Linker - Resin Conjugate (Figure 12)

43 *N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) β-D-glucopyranosyl amine - MBHA resin conjugate*

The resin from Example 38 was gently agitated with sodium methoxide (200 mg, 3.70 mmol) in abs. MeOH

(5 ml) at room temperature for 1 h. The resin was washed with abs. MeOH (5x10 ml), DMF(5x10 ml), ether (5x10 ml) and dried under high vacuum for 1 h, giving the resin-bonded unprotected β-D-glucopyranosyl amine. A sample of resin (5 mg) was cleaved by NH_3/MeOH (Example 41), and the resulting product was analyzed by TLC and mass spectometry, proving the quantitative deprotection.

Example 44 Synthesis of a Library of Di-, Tri- and Tetrasaccharides on a Solid Support (Figure 12)

44 *A mixture of mono-, di- and tri-O-(2,3,4-tri-O-benzyl α,β-L-fucopyranosyl)(1→2), (1→3), (1→4), (1→6)-[N-(6-(4,4-dimethyl-2,6-dioxocyclo-hexylidene)-hexanoic acid-6-yl)] β-D-glucopyranosyl amine - MBHA resin conjugate*

A mixture of resin from Example 43 and ethyl 2,3,4-tri-O-benzyl-1-thio-β-L-fucopyranoside (950 mg, 2 mmol) in dry CH_2Cl_2 (10 ml) was treated with dimethyl-(methylthio)-sulphonium trifluoromethanesulphonate (DMTST) (1.50 g, 5.81 mmol) at room temperature for 1 h. The resin was washed with dry CH_2Cl_2 (5x10 ml).

Example 45 Cleavage of a Library of Di-, Tri- and Tetrasaccharides from the Resin Affording Glycosyl Amine of Oligosaccharides (Figure 12)

45 *A mixture of mono-, di- and tri-O-(2,3,4-tri-O-benzyl α,β-L-fucopyranosyl)(1→2), (1→3), (1→4), (1→6)-β-D-glucopyranosyl amine*

The resin from Example 44 was treated with NH_3/MeOH (10 ml) for 5 min. The resin was filtered off, and the filtrate was evaporated giving a mixture of disaccharides, trisaccharides, and tetrasaccharides.

FAB MS disaccharides $C_{33}H_{41}NO_9$ (595.66), trisaccharides $C_{60}H_{69}NO_{13}$ (1012.16), tetrasaccharides $C_{87}H_{97}NO_{17}$ (1429.66)

(m/z (%) 618 $[M_{di}+Na]^+$ (41), 596 $[M_{di}+H]^+$ (57), 1034 $[M_{tri}+Na]^+$ (56), 1012 $[M_{tri}+H]^+$ (100), 1450 $[M_{tetra}+Na]^+$ (8), 1428 $[M_{tetra}+H]^+$ (10).

<u>Example 46</u> <u>Synthesis of a Second Sugar - Glycosyl Amine - Ddh Linker - Resin Conjugate</u> (Figure 13)

46 O-(2,3,6-tri-O-benzyl-4-O-bromoacetyl-α,β-D-galactopyranosyl)(1→4)-[N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl)] 2,3,6-tri-O-benzyl-β-D-galactopyranosyl amine - MBHA resin conjugate

A mixture of resin from Example 39 and ethyl 2,3,6-tri-O-benzyl-4-O-bromoacetyl-1-thio-β-D-galactopyranoside (1.25 g, 2 mmol) in dry CH_2Cl_2 (10 ml) was treated with dimethyl(methylthio)sulphonium trifluoromethanesulphonate (DMTST) (1.50 g, 5.81 mmol) at room temperature for 1 h. The resin was washed with dry CH_2Cl_2 (5x10 ml).

<u>Example 47</u> <u>Selective Deprotection of the Second Sugar - Glycosyl Amine - Ddh Linker - Resin Conjugate</u> (Figure 13)

47 O-(2,3,6-tri-O-benzyl-α,β-D-galacto-pyranosyl)(1→4)-[N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl)] 2,3,6-tri-O-benzyl-β-D-galactopyranosyl amine - MBHA resin conjugate

The resin from Example 46 was gently agitated with sodium methoxide (200 mg, 3.70 mmol) in abs. MeOH (5 ml) at room temperature for 1 h. The resin was washed with abs. MeOH (5x10 ml), DMF(5x10 ml), ether (5x10 ml) and dried under high vacuum for 1 h, giving the resin bonded partially unprotected disaccharide. A sample of resin (5 mg) was cleaved by NH_3/MeOH (Example 41) and the resulting product was analyzed by TLC and mass spectometry, proving the quantitative deprotection.

Example 48 Cleavage of a Second Sugar - Glycosyl Amine - Ddh Linker - Resin Conjugate Affording a Glycosyl Amine of a Disaccharide (Figure 13)

48 O-(2,3,6-tri-O-benzyl-α,β-D-galacto-pyranosyl)(1→4)-2,3,6-tri-O-benzyl-β-D-galactopyranosyl amine

The resin from Example 47 was treated with NH_3/MeOH (10 ml) for 5 min. The resin was filtered off, and the filtrate was evaporated giving an anomeric mixture of disaccharides. FAB MS $C_{54}H_{59}NO_{10}$ (882.01) (m/z (%) 904 $[M+Na]^+$ (100), 880 $[M+H]^+$ (41).

Example 49 Cleavage of a Carbohydrate - Ddh-Aminobenzyl Linker - Resin Conjugate Affording an Aminobenzyl Protected Carbohydrate (Figure 14)

49 4-aminobenzyl β-D-glucopyranoside

The resin from Example 29 was treated with NH_3/MeOH (5 ml) overnight. The resin was filtered off, and the filtrate was evaporated giving 4-aminobenzyl β-D-glucopyranoside.

R_f 0.55 ($CHCl_3$/MeOH/H_2O 10:4:0.5 v/v/v); FAB MS $C_{13}H_{19}NO_5$ (269.28) m/z (%) 402 $[M+Cs]^+$ (25), 292 $[M+Na]^+$ (50), 270 $[M+H]^+$ (18).

Example 50 Deprotection of 4-Aminobenzyl Protected Carbohydrate (Figure 14)

50 β-D-Glucopyranose

4-Aminobenzyl β-D-glucopyranoside (110 mg, 0.40 mmol) was hydrogenated over Pd/C (10%) (50 mg) in MeOH (5 ml) at room temperature for 4 hours. The catalyst was filtered off and the filtrate was evaporated affording D-glucose in quantitative yield.

Example 51 Immobilization of an Oligosaccharide (Figure 15)

51 O-[O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl(1→4))-2,3,6-tri-O-acetyl-β-D-glucopyranosyl(1→4)]-2,3,6-tri-O-acetyl-β-D-glucopyranosyl amine using 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid - MBHA resin conjugate

The resin from Example 37 was reacted with O-[O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl(1→4))-2,3,6-tri-O-acetyl-β-D-glucopyranosyl(1→4)]-2,3,6-tri-O-acetyl-β-D-glucopyranosyl amine (1.80 g, 2.00 mmol) in DMF (5 ml) at room temperature for 2 days. The resin was washed with DMF (5x10 ml).

Example 52 Synthesis of an aminosugar - Ddh - resin conjugate (Figure 16)

52 N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid-6-yl) D-glucosamine - MBHA resin conjugate

A mixture of resin from Example 37 and glucosamine (350 mg, 2 mmol) in DMF (20 ml) was stirred at room temperature for 2 days. The resin was filtered off, washed with DMF/H_2O 4:1 (5x10 ml), DMF 5x10 ml, MeOH (5x10), ether (5x10 ml), and dried under high vacuum overnight.

It will be apparent to the person skilled in the art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and methods described herein may be made without departing from the scope of the inventive concept disclosed in this invention.

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CLAIMS

1. A support for solid-phase synthesis of oligosaccharides, said support comprising

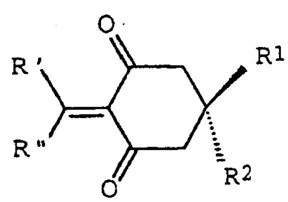
a) a resin,

b) a linker covalently attached to the resin, and

c) one or more saccharide groups covalently attached to the resin via the linker,

wherein the linker is a 2-substituted-1,3-dioxocycloalkane compound, and

said support having general formula I



I

in which

R^1 and R^2 may be the same or different, and is each hydrogen or C_{1-4} alkyl; preferably both R^1 and R^2 are methyl;

R' is an amino sugar, a glycosylamine, or a glycosylamine of an oligosaccharide; a mono or oligosaccharide coupled through an alkyl-, substituted alkyl-, aryl-, substituted aryl-, cycloalkyl-, or substituted cycloalkyl-amino group; or a mono or oligosaccharide coupled through a carboxyalkyl-, substituted carboxyalkyl-, carboxyaryl-, substituted carboxyaryl-, carboxycycloalkyl-, or substituted carboxycycloalkyl-amino group, and

R" is an alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, or substituted cycloalkyl spacer group which is directly coupled to the resin support, or which may optionally be coupled to the resin

support via a covalent linkage which is stable to conditions of oligosaccharide synthesis and cleavage.

2. A support according to Claim 1, in which both R^1 and R^2 are methyl.

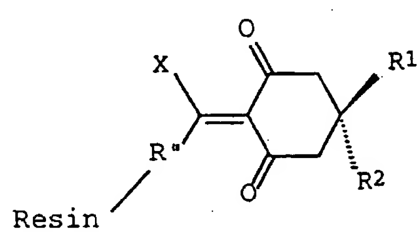
3. A support according to Claim 1 or Claim 2, in which R' is an oligosaccharide-O-CH_2-(C_6H_4)-NH, monosaccharide-O-CH_2-(C_6H_4)-NH, amino-oligosaccharide-CO_2CH_2-(C_6H_4)NH, or amino-monosaccharide-CO_2CH_2-(C_6H_4)-NH group.

4. A support according to any one of Claims 1 to 3, in which the covalent linkage to the resin is provided by a -CONH-, -O-, -S-, -COO-, -CH=N-, -NHCONH-, -NHCSNH, or -NHNH- grouping.

5. A support according to any one of Claims 1 to 4, in which the linker is functionalised Dde, Ddh or ODMab.

6. A support according to any one of Claims 1 to 5, comprising a resin, a linker and a monosaccharide, an oligosaccharide, an aminosaccharide or an amino-oligosaccharide.

7. A support for solid-phase synthesis comprising a resin and a linker group, wherein the linker is a 2-substituted-1,3-dioxocycloalkane of general formula II:



II

in which

X is OH or NH_2;

R^1 and R^2 may be the same or different, and is each hydrogen or C_{1-4} alkyl; and

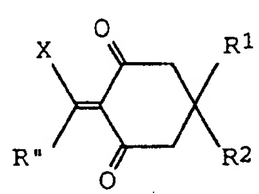
R" is an alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, or substituted cycloalkyl spacer group which is directly coupled to the resin support, or which may optionally be coupled to the resin support via a covalent linkage which is stable to conditions of oligosaccharide synthesis and cleavage.

8. A support according to Claim 7, in which R^1 and R^2 are both methyl

9. A support according to Claim 7 or Claim 8, in which the covalent linkage to the resin is provided by a -CONH-, -O-, -S-, -COO-, -CH=N-, -NHCONH-, -NHCSNH, or -NHNH- grouping.

10. A linker-saccharide complex in which the linker group is as defined in Claim 1 or Claim 2 and the saccharide is as defined in Claim 1 or Claim 6.

11. A compound carrying functional groups suitable to attach a primary amine to a resin via covalent bonds which are stable to conditions of oligosaccharide synthesis and cleavage, said compound having general formula III



III

in which

X is OH or NH_2;

R^1 and R^2 may be the same or different, and is each hydrogen or C_{1-4} alkyl, and

R" is an alkyl, substituted alkyl, aryl, substituted aryl, cycloalkyl, or substituted cycloalkyl spacer group, which carries a functionality capable of reacting with a functionalised resin.

12. A compound according to Claim 11, in which both R^1 and R^2 are methyl.

13. A compound according to Claim 11 or Claim 12, in which the functionality on R" is a carboxyl group.

14. A compound according to Claim 11, which is 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-hexanoic acid or an ester thereof.

15. A compound according to Claim 14, in which the ester is a benzyl, methyl or t-butyl ester.

16. A support according to any one of Claims 1 to 6, in which the linker is a compound according to any one of Claims 11 to 15.

17. A support according to any one of Claims 7 to 9, in which the linker is a compound according to any one of Claims 11 to 15.

18. A linker-saccharide complex according to Claim 10, in which the linker is a compound according to any one of Claims 11 to 15.

19. A kit for solid phase synthesis or combinatorial synthesis of oligosaccharides, comprising:

a) a resin-linker-saccharide support according to any one of Claims 1 to 5 or 16,

b) a linker-saccharide complex according to Claims 10 or 17, or

c) a resin-linker support according to any one of Claims 7 to 17,

and optionally also comprising one or more protecting agents, deprotecting agents, and/or solvents suitable for solid phase or combinatorial synthesis.

20. A method of solid-phase synthesis of oligosaccharides, comprising the step of sequentially linking mono- or oligosaccharide groups to a support as defined in any one of Claims 1 to 9 or 16.

21. A method of synthesis of a linker group according to general formula I as defined in Claim 1, comprising the step of C-acylation of a 2-substituted 1,3-dioxocyclohexane compound with a dicarboxylic acid, and

optionally reacting the product of the C-acylation reaction with 4-aminobenzyl alcohol, to form the 4-aminobenzyl derivative.

22. A method according to Claim 21, in which the dicarboxylic acid is mono-protected by ester formation.

23. A method according to Claim 21 or Claim 22, in which the C-acylation reaction is activated with carbodiimide and catalysed by N,N'-dimethylaminopyridine.

24. A method of synthesis of a resin-linker support according to any one of Claims 6 to 9, comprising the step of swelling a resin in a suitable solvent, treating the swollen resin with a dicarboxylic acid, and reacting the thus-produced product with a 2-substituted 1,3-dioxocycloalkane compound.

25. A method according to any one of Claims 21 to 24, in which the 2-substituted 1,3-dioxocycloalkane compound is 5,5-dimethyl-1,3-cyclohexanedione.

26. A method according to any one of Claims 21 to 25, in which the dicarboxylic acid is adipic acid.

27. A support according to claim 1 or claim 7, substantially as herein described with reference to the examples and drawings.

28. A compound according to claim 11, substantially as herein described with reference to the examples and drawings.

Dated this 10th day of October 2000

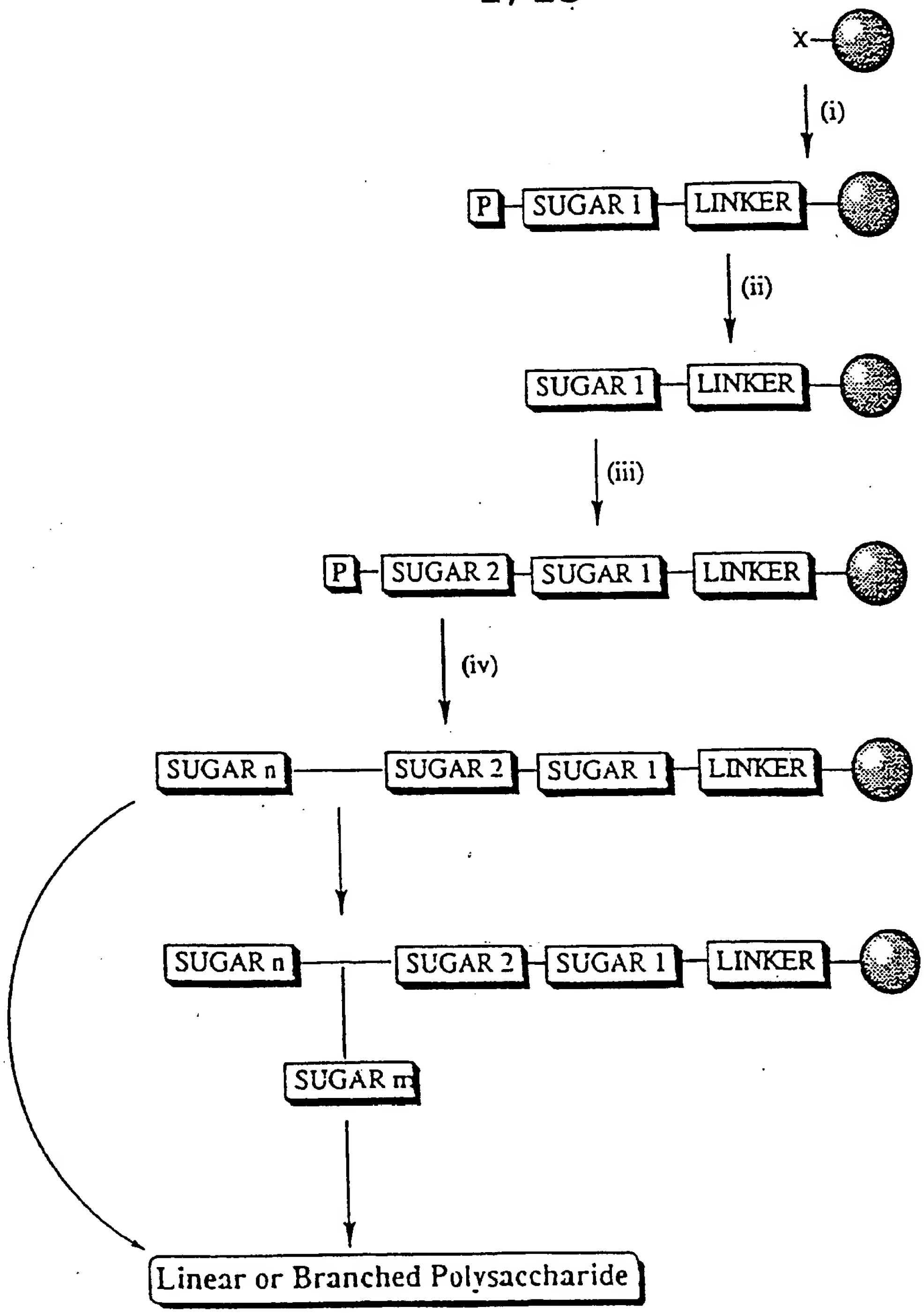
ALCHEMIA PTY LTD

By their Patent Attorneys

GRIFFITH HACK

Fellows Institute of Patent and

Trade Mark Attorneys of Australia



Conditions: (i) Attachment of sugar-linker conjugate to a resin support.
(ii) Selective deprotection of one sugar hydroxyl group.
(iii) Coupling of next sugar residue.
(iv) Repeat of steps (ii) and (iii) as desired.

FIGURE 1

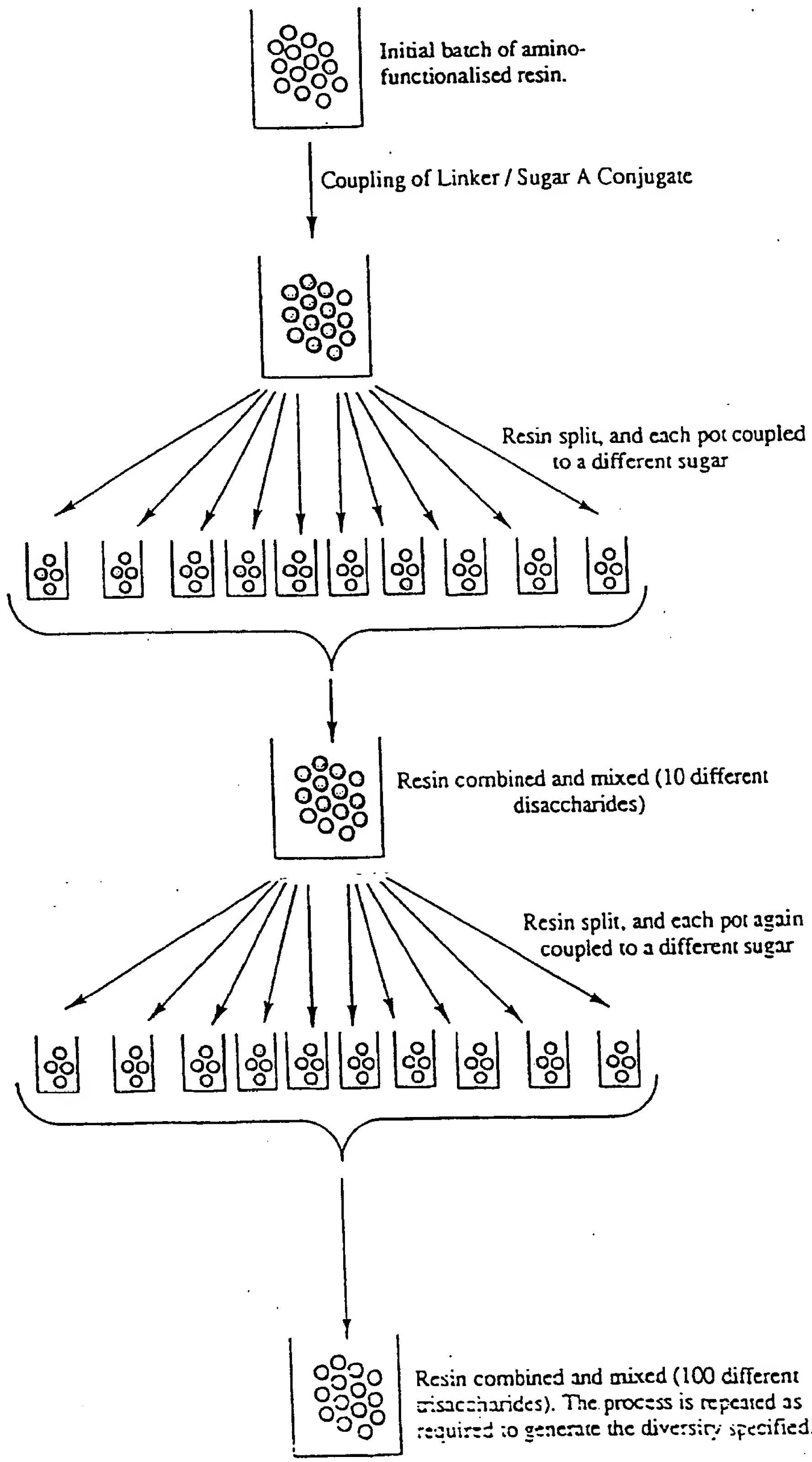

Initial batch of amino-functionalised resin.
Coupling of Linker / Sugar A Conjugate
Resin split, and each pot coupled to a different sugar
Resin combined and mixed (10 different disaccharides)
Resin split, and each pot again coupled to a different sugar
Resin combined and mixed (100 different disaccharides). The process is repeated as required to generate the diversity specified.

FIGURE 2

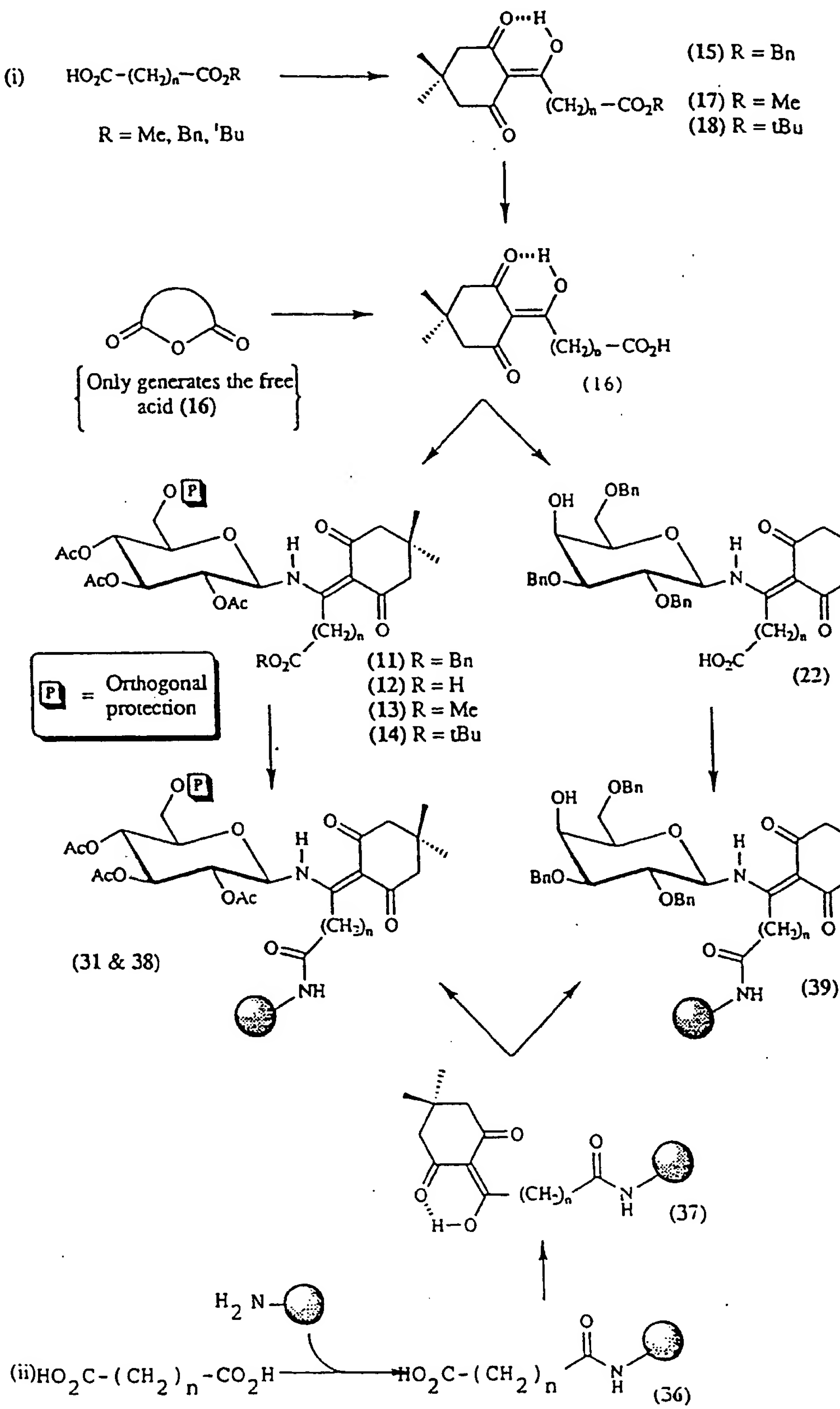

(i) $HO_2C-(CH_2)_n-CO_2R$
R = Me, Bn, ᵗBu
(15) R = Bn
(17) R = Me
(18) R = tBu
Only generates the free acid (16)
(16)
(11) R = Bn
(12) R = H
(13) R = Me
(14) R = tBu
(22)
P = Orthogonal protection
(31 & 38)
(39)
(37)
(ii) $HO_2C-(CH_2)_n-CO_2H$
(36)

FIGURE 3

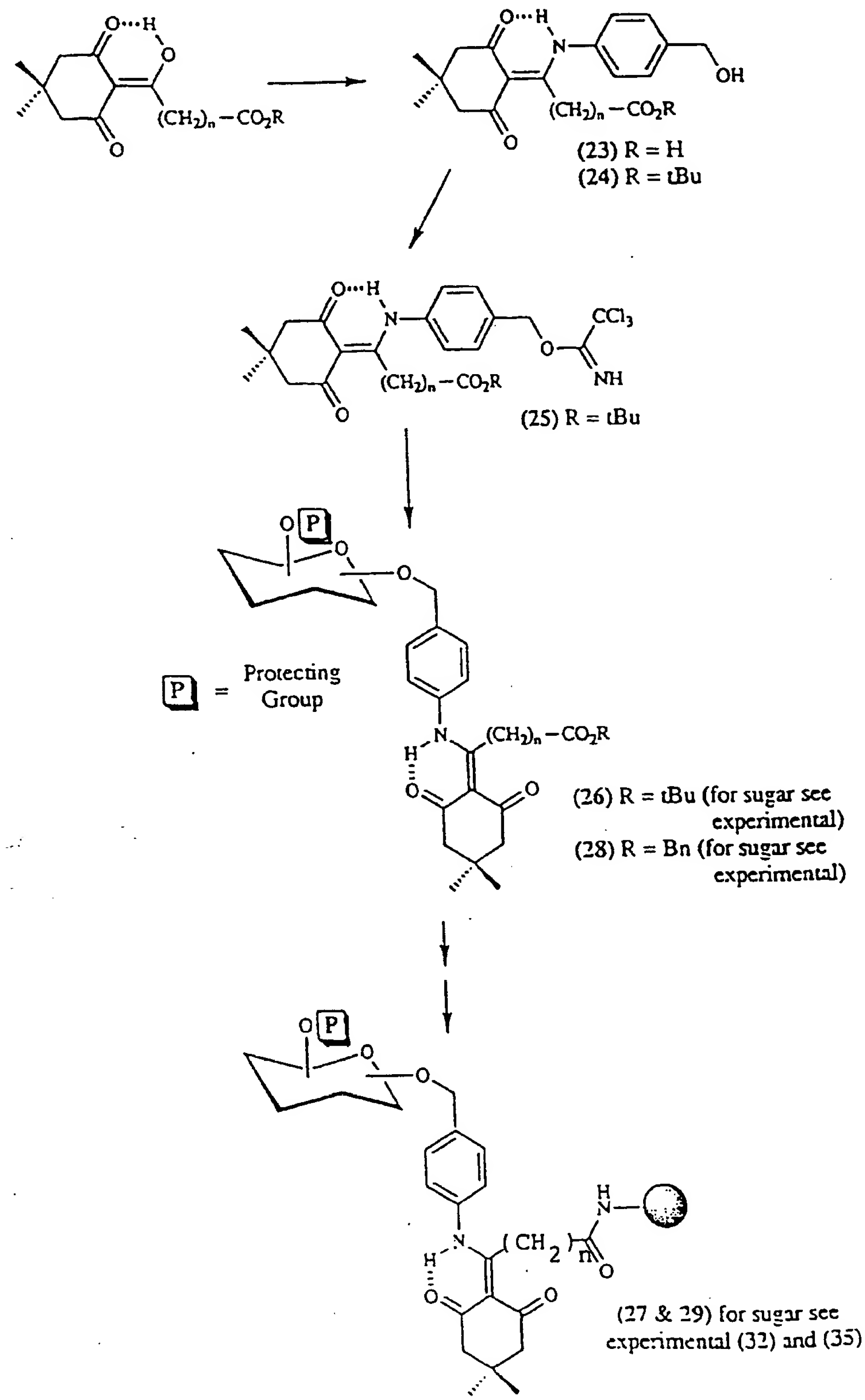
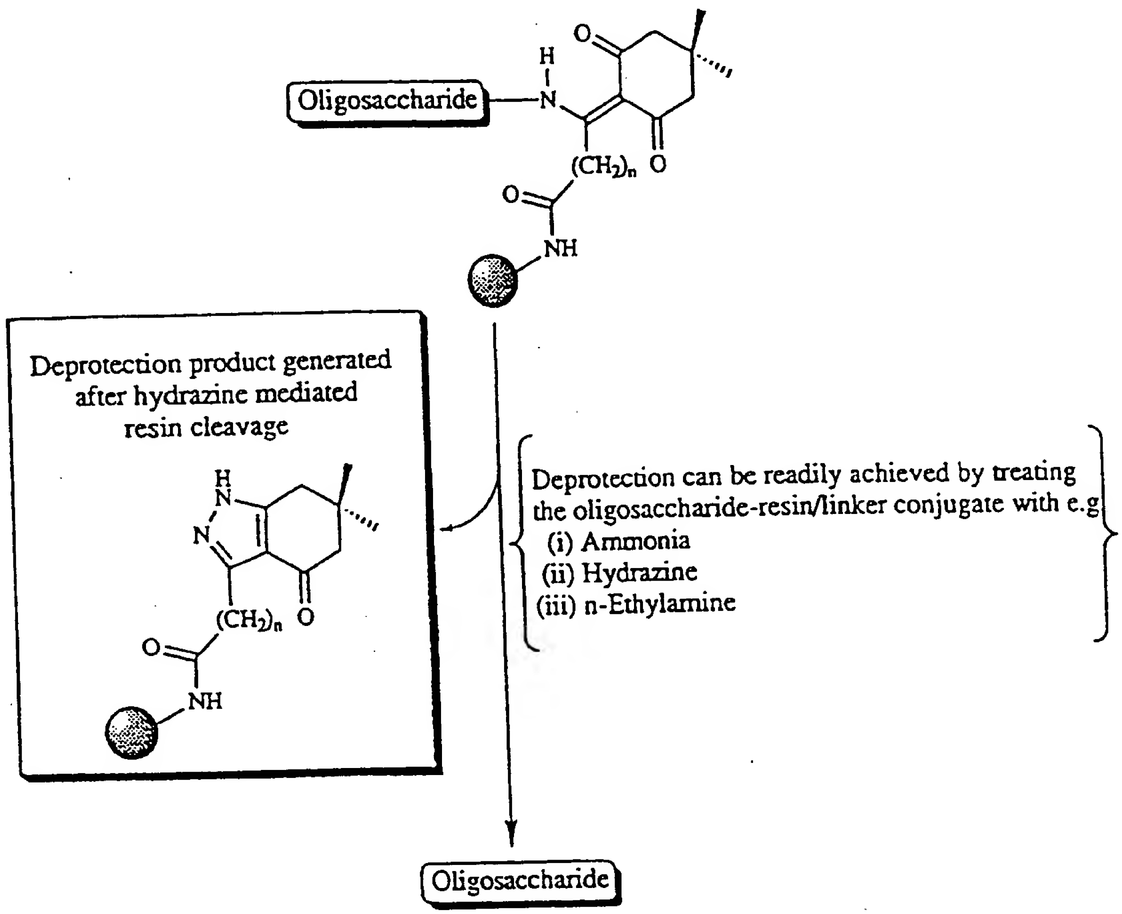
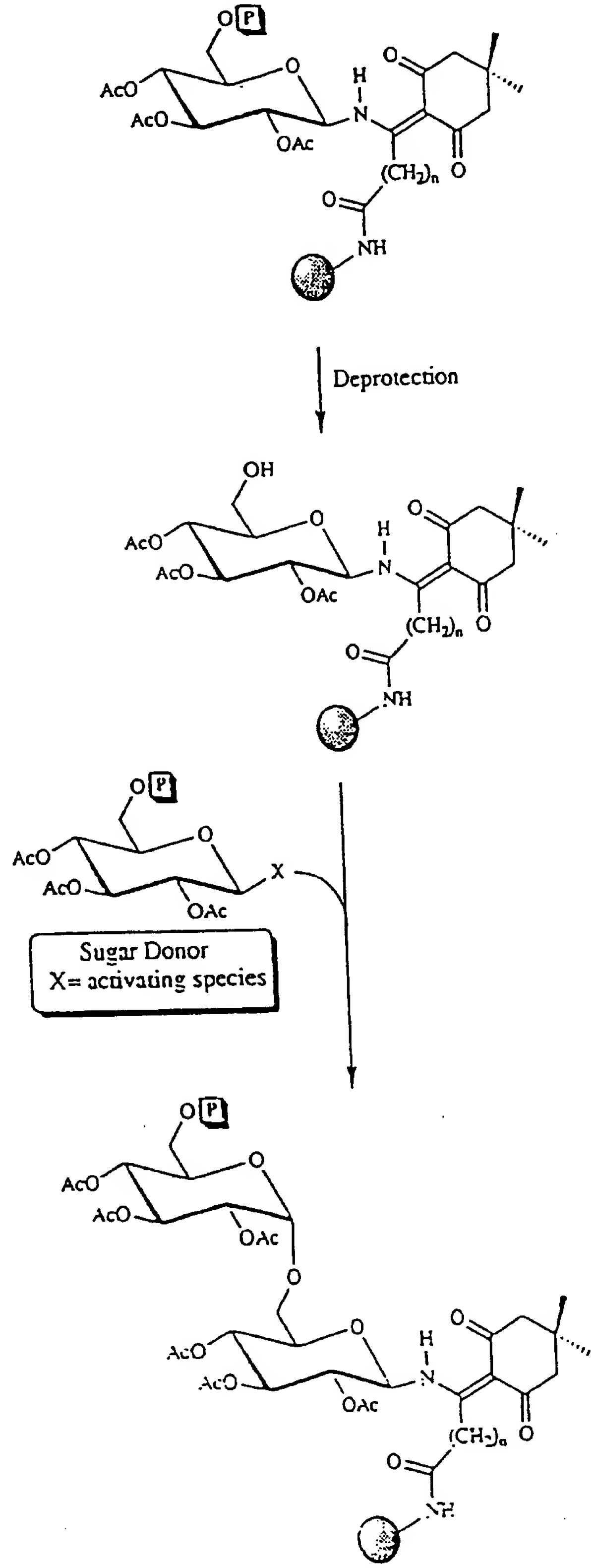

(23) R = H
(24) R = tBu
(25) R = tBu
P = Protecting Group
(26) R = tBu (for sugar see experimental)
(28) R = Bn (for sugar see experimental)
(27 & 29) for sugar see experimental (32) and (35)

FIGURE 4



NB. The Oligosaccharide can potentially be released in either the protected or deprotected form depending on the choice of monomer protection employed during the synthesis.

FIGURE 5



AcO
AcO
OAc
O
H
N
(CH2)n
O
NH
Deprotection
OH
X
Sugar Donor
X= activating species

FIGURE 6

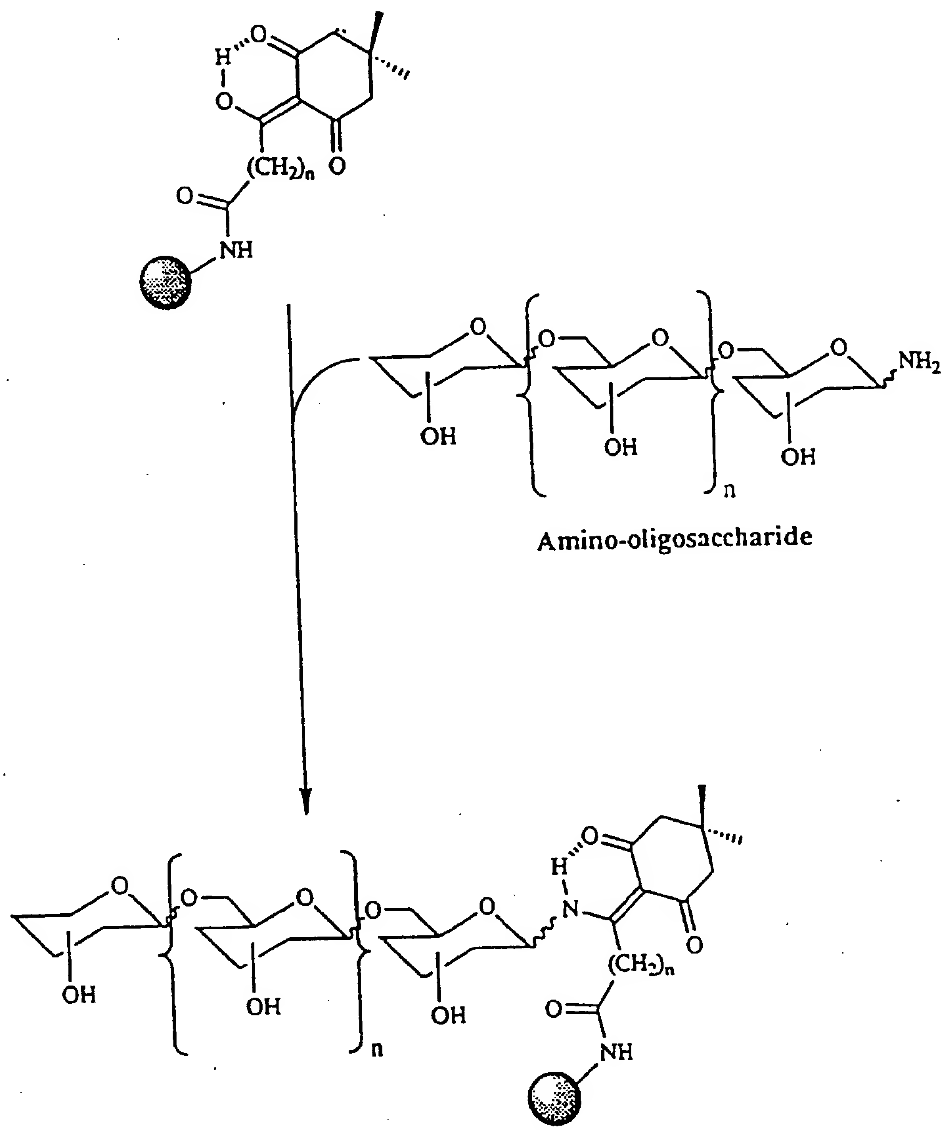


FIGURE 7

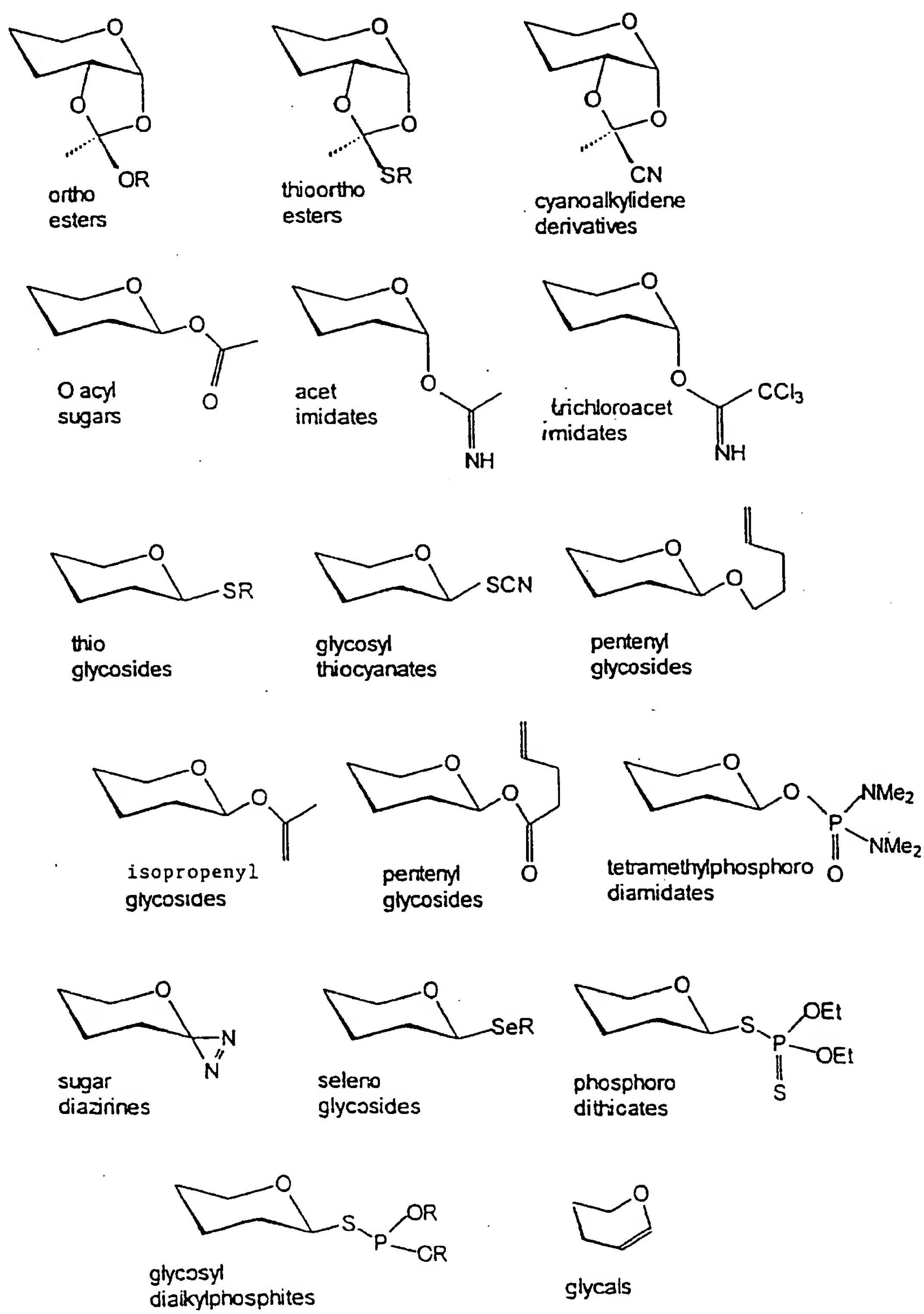
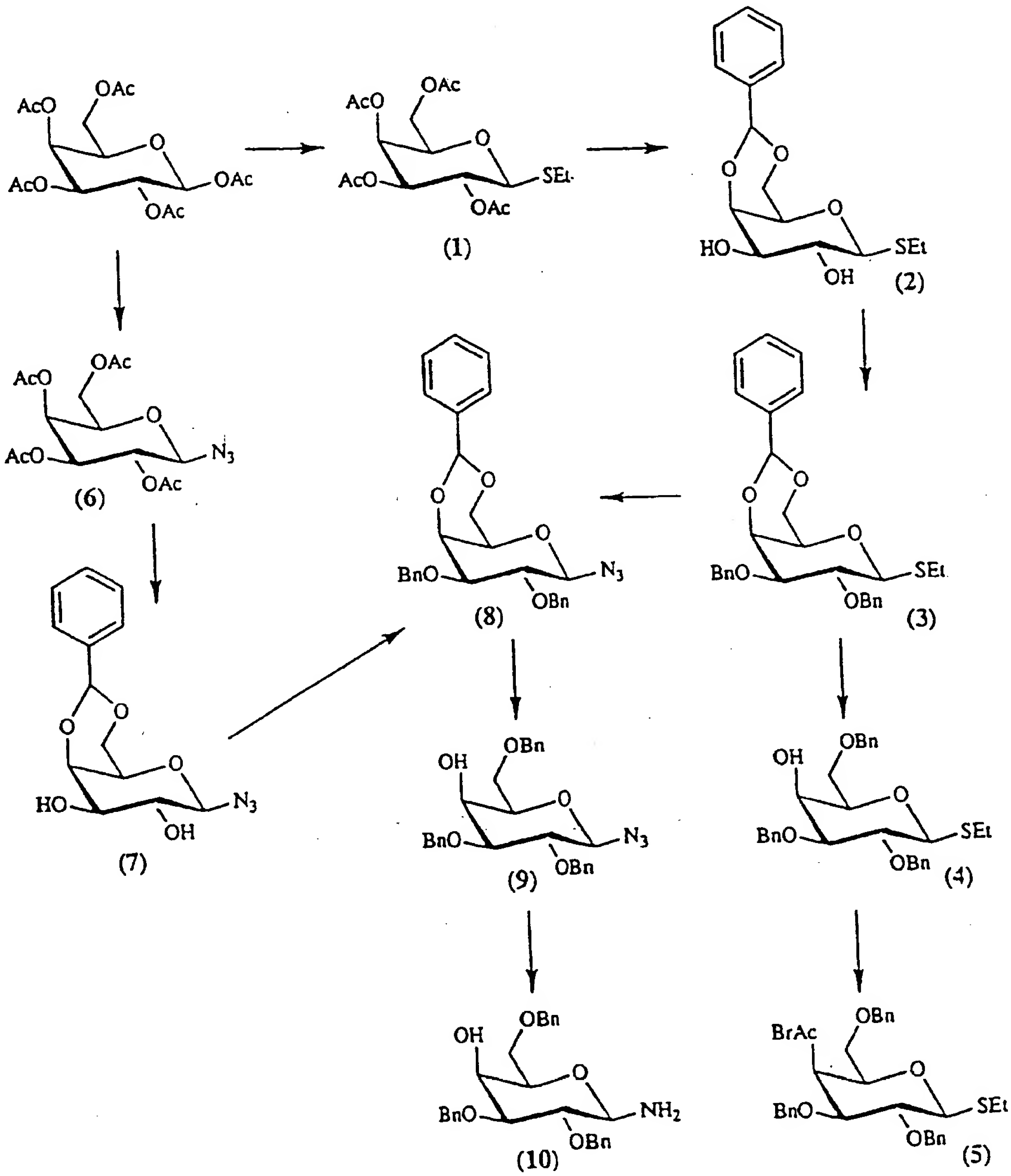

OR
ortho esters
SR
thioortho esters
CN
cyanoalkylidene derivatives
O acyl sugars
acet imidates
NH
trichloroacet imidates
CCl3
NH
SR
thio glycosides
SCN
glycosyl thiocyanates
pentenyl glycosides
isopropenyl glycosides
pentenyl glycosides
NMe2
NMe2
tetramethylphosphoro diamidates
N
N
sugar diazirines
SeR
seleno glycosides
OEt
OEt
phosphoro dithioates
OR
OR
glycosyl dialkylphosphites
glycals

FIGURE 8



AcO
OAc
O
AcO
OAc
OAc
SEt
(1)
HO
OH
(2)
N3
(6)
BnO
OBn
(8)
(3)
(7)
OH
OBn
(9)
(4)
NH2
(10)
BrAc
(5)

FIGURE 9

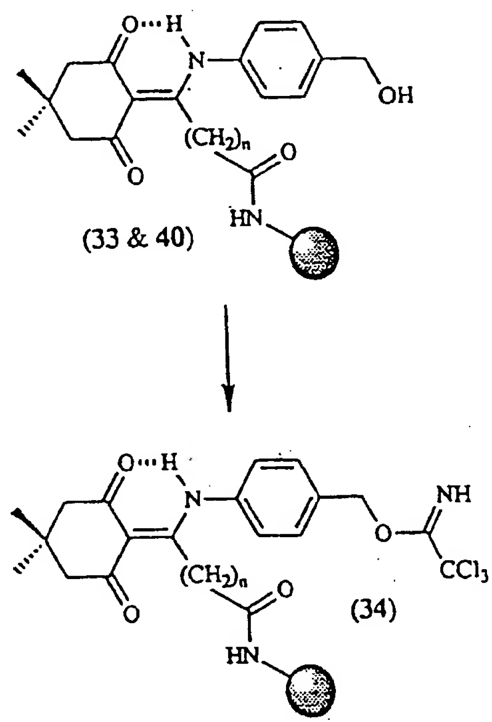
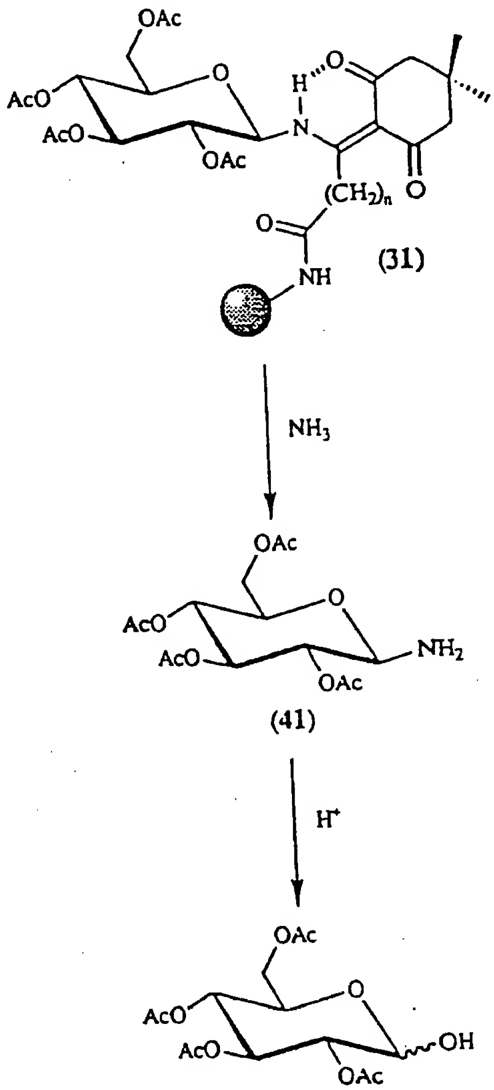


FIGURE 10



(42)

FIGURE 11

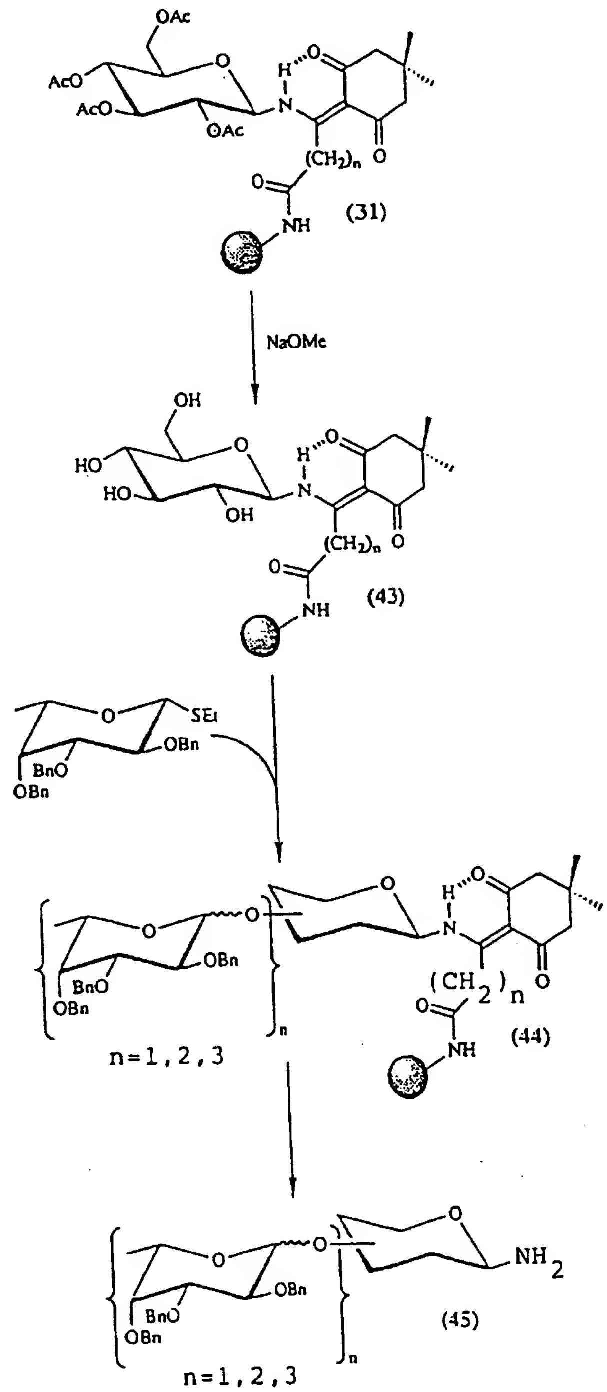

OAc
AcO
AcO
OAc
H
N
O
(CH2)n
O
O
NH
(31)
NaOMe
OH
HO
HO
OH
(43)
SEt
OBn
BnO
OBn
(44)
n=1,2,3
NH2
(45)
n=1,2,3

FIGURE 12

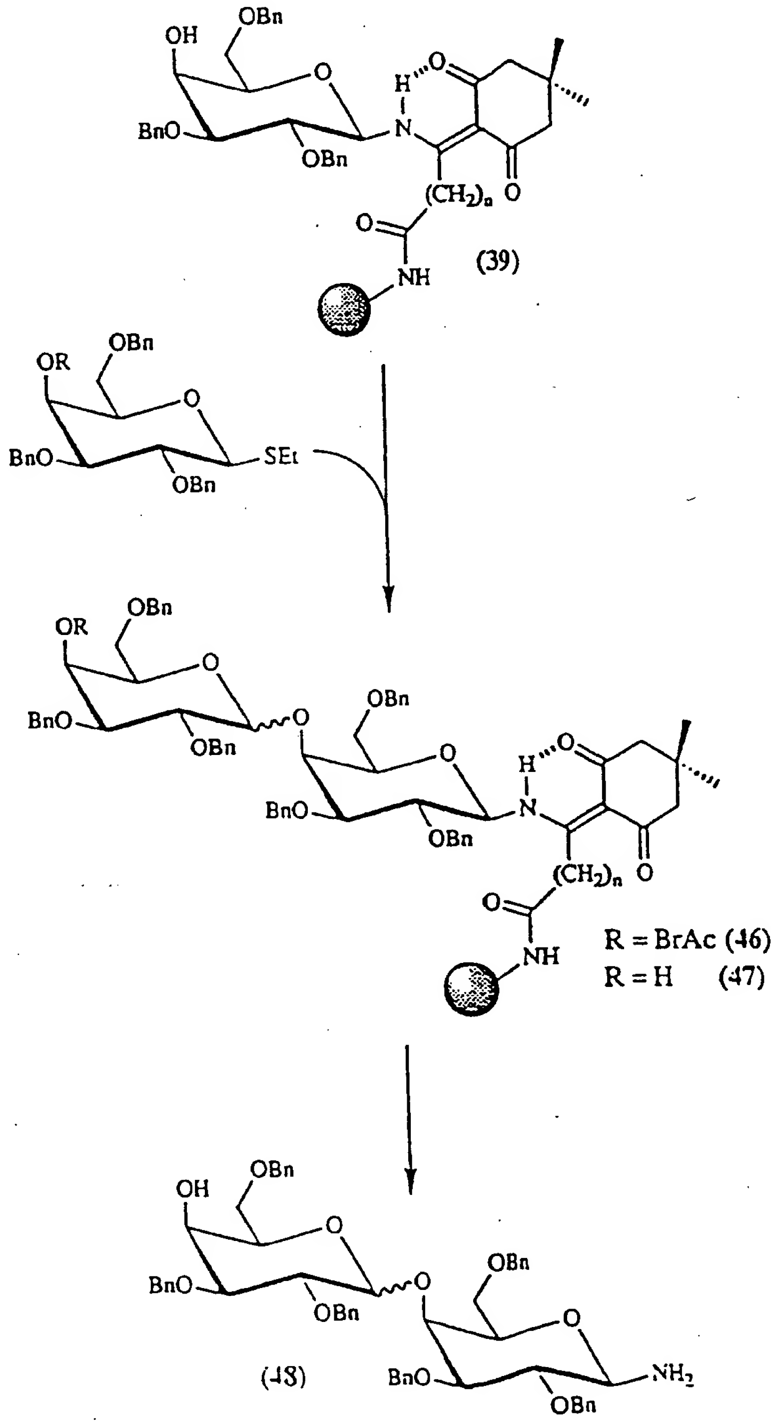
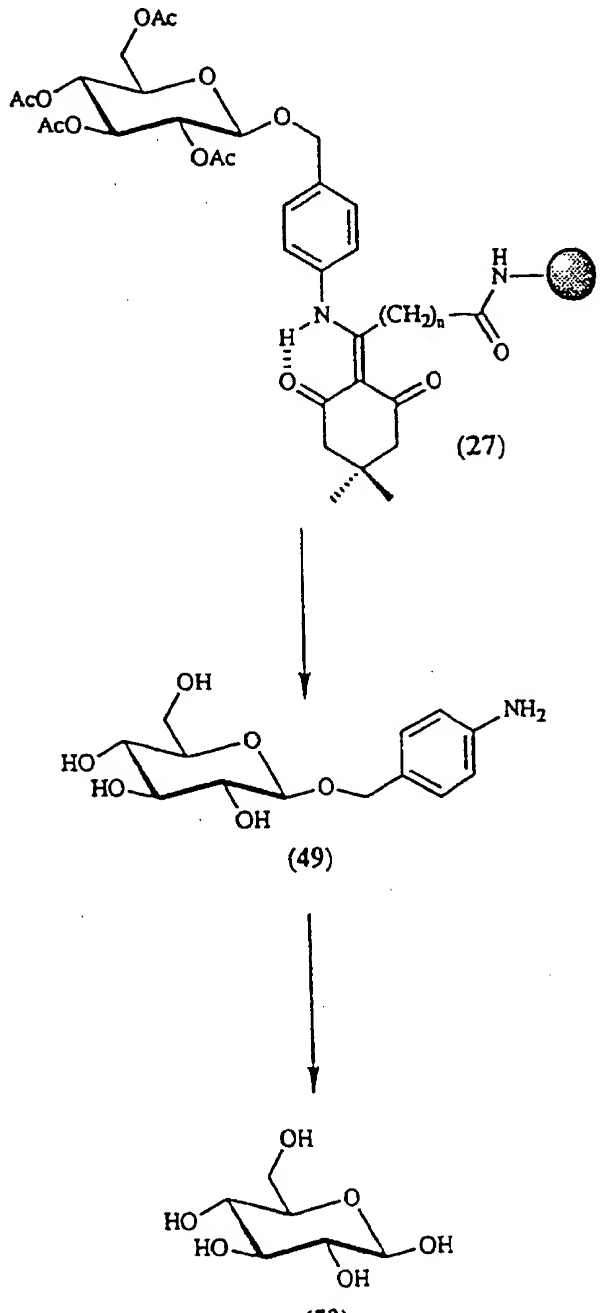


FIGURE 13



(27)

(49)

(50)

FIGURE 14

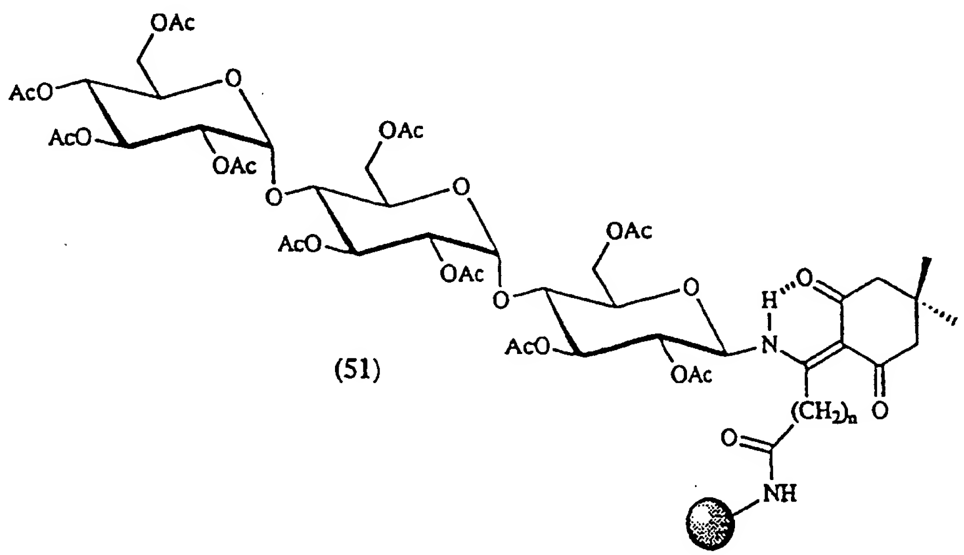


FIGURE 15

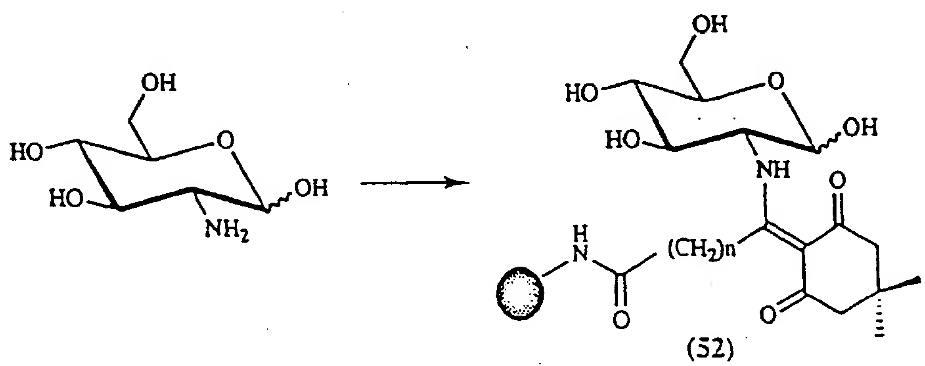


FIGURE 16